(51) International Patent Classification [6] :		(11) International Publication Number: **WO 95/35102**
A61K 31/00, C07H 1/00, 19/00, 21/00	**A1**	(43) International Publication Date: 28 December 1995 (28.12.95)

(21) International Application Number: PCT/US95/06641

(22) International Filing Date: 25 May 1995 (25.05.95)

(30) Priority Data:
08/264,029   22 June 1994 (22.06.94)   US

(60) Parent Application or Grant
(63) Related by Continuation
US   08/264,029 (CIP)
Filed on   22 June 1994 (22.06.94)

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(81) Designated States: AM, AT, AU, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, HU, JP, KE, KG, KP, KR, KZ, LK, LR, LT, LU, LV, MD, MG, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SI, SK, TJ, TT, UA, US, UZ, VN, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG), ARIPO patent (KE, MW, SD, SZ, UG).

Published
With international search report.

(54) Title: NOVEL METHOD OF PREPARATION OF KNOWN AND NOVEL 2'-MODIFIED NUCLEOSIDES BY INTRAMOLECULAR NUCLEOPHILIC DISPLACEMENT

(57) Abstract

2'-modified pyrimidines are prepared by a novel intramolecular nucleophilic substitution reaction.

NOVEL METHOD OF PREPARATION OF KNOWN AND NOVEL 2'-MODIFIED NUCLEOSIDES BY INTRAMOLECULAR NUCLEOPHILIC DISPLACEMENT

5   **FIELD OF THE INVENTION**

The present invention relates to methods of production of modified nucleosides. In particular, this invention includes novel methods for the production of 2'-modified pyrimidines. The preparation

10   of such 2'-modified pyrimidines is accomplished in a novel intramolecular nucleophilic displacement reaction. Also included within the scope of this invention are certain novel 2'-modified pyrimidines prepared according to the method of the invention, and

15   oligonucleotides containing such modified pyrimidine species. 2'-modified purines are also prepared by the method of the invention. The 2'-modified nucleosides are also useful as anti-viral and anti-neoplastic agents.

20

BACKGROUND OF THE INVENTION

Modified nucleotides and oligonucleotides have gained an important role in the development of pharmaceuticals over the last several years. For

25   example, analogs of nucleosides and nucleotides have been employed as antiviral compounds. Oligonucleotides comprised of nucleotide analog building blocks have been used as inhibitors of gene translation. (*See*, Huryn and Okabe (1992) Chem. Rev. 92:1745-1788).

30   The recent discovery of oligonucleotide library screening technology has opened up an additional area for the pharmaceutical application of nucleotide analogs in oligonucleotides; as highly specific, high

affinity inhibitors of protein function. *See*, e.g.,
U.S. patent number 5,270,163 entitled, Nucleic Acid
Ligands; and Tuerk and Gold (1990) Science 249:505-510.
This technology is called SELEX, an acronym for

5   Systematic Evolution of Ligands by Exponential
Enrichment. SELEX can be carried out with libraries
comprised of modified oligonucleotides to give ligands
incorporating desired chemical functionalities (*See*,
U.S. patent application serial number 08/117,991, filed

10   September 8, 1992 and entitled "High Affinity Nucleic
Acid Ligands containing Modified Nucleotides").

Stability against nuclease degradation is a
concern in the field of oligonucleotide therapeutics.
Oligodeoxynucleotides are often stabilized by the

15   introduction of phosphorothioate internucleotidic
linkages. (*See*, Huryn and Okabe (1992) Chem. Rev.
92:1745-1788; Englisch and Gauss (1991) Angew. 30:613-
722).

Modification of the 2'-position of pyrimidines has

20   also been shown to stabilize oligonucleotides against
nuclease degradation. (*See*, Paolella *et al.* (1992)
EMBO Journal 11:1913-1919; Pieken *et al.* (1991) Science
253:314-317.) Both 2'-amino and 2'-fluoro nucleotides
have been used for this purpose. The 5'-triphosphate

25   derivatives of these modified nucleotides are
substrates for T7 RNA polymerase. (Aurup and Eckstein
(1992) Biochemistry 31:9636-9641.)

The introduction of modifications to the 2'-
position of pyrimidine nucleosides is not a highly

30   efficient process. Furthermore, current technology
allows only for the preparation of a few select 2'-

modified pyrimidines under harsh reaction conditions
with low yield. (Verheyden *et al.* (1971) J. Org. Chem.
36:250-254.) A general reaction allowing facile
preparation of a wide variety of novel and known 2'-
5   modified pyrimidines has to date not been available.

To facilitate incorporation into oligonucleotide
libraries, nucleotide analogs have to be prepared as
the 5'-triphosphate derivatives. This is the form that
is recognized as a substrate for DNA dependent RNA
10   polymerases. Furthermore, analogs also have to be
prepared as the phosphoramidites in order to be
incorporated into the final oligonucleotide ligand by
automated chemical synthesis.

There currently is no reliable method for the
15   stereoselective preparation of ribo-2'-hydroxyl-
aminopyrimidines. Derivatives of such compounds have
not been described nor have these compounds been
characterized. It has been reported that the BH_3
reduction of the oxime derivative of 2'-ketouridine
20   affords mostly the 2'-hydroxylaminonucleosides of the
arabino configuration. (Tronchet *et al.* (1990)
Tetrahedron Lett. 31:531.) 2'-Halomethylpyrimidines
are unknown. The 2'-aminopyrimidines are known
compounds, however, they have never been prepared by an
25   intramolecular introduction of the amino group. All
previous procedures for synthesizing such compounds
have proceeded through the 2'-azido precursor. (*See*,
Verheyden *et al.* (1971) J. Org. Chem. 36:250-254.)

Cyclization reactions where a neighboring hydroxyl
30   group is exploited as an anchor for a nucleophile which
is then positioned to undergo cyclization with

concomitant opening of an existing heterocycle have
been observed in the opening of epoxyalcohols. (Jung
and Jung (1989) Tetrahedron Lett. 30:6637-6640.)

5

10

A   B   C
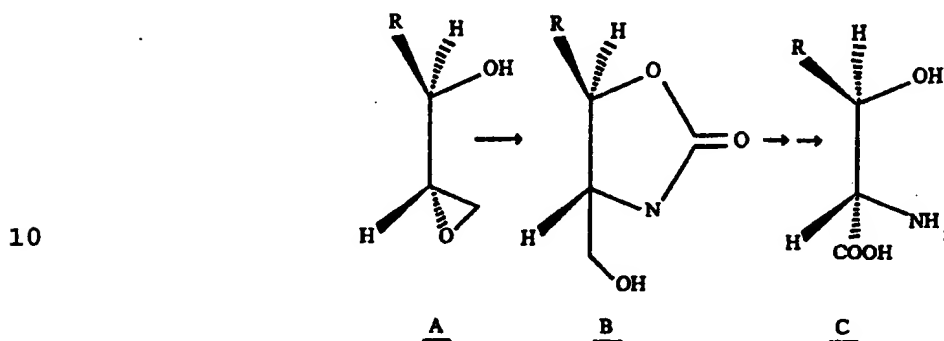
15   Oxidation and hydrolysis of B gave the desired β-
hydroxy α-amino acid C. It is not obvious that the
crucial cyclization step should work analogously to
open 2,2' anhydropyrimidines. Furthermore, the
reported hydrolysis conditions are incompatible with
20   nucleosides.

Other examples of similar intramolecular openings
of epoxides have been reported. Roush et al. showed
that a phenylcarbamate group can be directed to open an
adjacent epoxide through the carbamate nitrogen or
25   oxygen, depending on the reaction conditions. (See,
Roush and Brown (1983) J. Org. Chem. 48:5093; Roush and
Adam (1985) J. Org. Chem. 50:3752.) Many additional
examples of intramolecular nucleophilic epoxy alcohol
ring openings by carbon, nitrogen, oxygen, and sulfur
30   nucleophiles, as well as reductive openings and
chelated additions by organometallic species have been

reported. (*See,* **Intramolecular carbon nucleophilic
openings of epoxy alcohols:** McCombie *et al.* (1985)
Tetrahedron Lett. 26:6301; McCombie *et al.* (1989)
Tetrahedron Lett. 30:7029. **Bicyclic products** (e.g.;
epoxides of cyclic olefins) Padwa *et al.* (1991) J. Org.
Chem. 56:3556. **Epoxy alcohol conversions by
organometallic reagents:** Me₂CuLi and/ or Me₃Al: Roush
et al. (1983) Tetrahedron Lett. 24:1377; Hishikubo and
Kishi, (1981) Tetrahedron 37:3873; Johnson *et al.*
(1979) Tetrahedron Lett. 20:4343. **Ti(OiPr)₄-Mediated:**
Caron and Sharpless, (1985) J. Org. Chem. 50:1557.
Reductive openings of epoxy alcohols: Red-Al/ DiBAl:
Finan and Kishi, (1982) Tetrahedron Lett. 23:2719; Viti
(1982) Tetrahedron Lett. 23:4541; Katsuki *et al.* (1982)
J. Org. Chem. 47:1378; Nicolaou and Uenishi (1982) J.
Chem. Soc. Chem. Common. 1292. **Intramolecular nitrogen
nucleophilic openings of epoxy alcohols: N-Hydroxyl
amides:** Roush and Follows (1994) Tetrahedron Lett.
35:4935. **Alkyl and acyl carbamates:** Knapp *et al.* (1987)
Tetrahedron Lett. 28:5399; McCombie and Nagabhushan,
ibid 5395; Roush and Adam (1985) J. Org. Chem. 50:3752;
Roush and Brown, *ibid,* (1982) 47:1371; Minami *et al.*
(1982) J. Amer. Chem Soc. 104:1109. **Bicyclic products**
(e.g.; epoxides of cyclic olefins): Schubert, *et al.*
(1986) Liebigs Annalen der Chemie 2009. **Intramolecular
oxygen nucleophilic openings of epoxy alcohols:**
McCombie and Metz (1987) Tetrahedron Lett. 28:383;
Roush *et al.* (1983) J. Org. Chem. 48:5093; Katsuki *et
al.* (1982) J. Org. Chem. 47:1373. **The opening of an
epoxide by an adjacent trichloroacetimidate has been
reported:** Bernet and Vas lla, (1983) Tet. Letters

49:5491-5494.

2'-O-Methyl ethers of nucleosides are known to occur in nature as minor components of transfer RNA. (R. H. Hall, " The Modified Nucleosides in Nucleic Acids." Columbia University Press, New York, NY, 1971). 2'-O-Alkyl substituted nucleosides have been used to stabilize oligonucleotides against chemical and enzymatic degradation (For example see E. DeClerq et al., FEBS Letters, 1972, 24, 137; H. Inoue et. al., FEBS Letters, 1987, 215, 327; A. M. Iribarren et. al. , Proc. Natl. Acad. Sci. USA 1990, 87, 7747; G. Kawai et. al., Biochemistry 1992, 31, 1040.) . 2'-O-Alkyl substitutents also can serve as removable protecting groups for the 2'-hydroxyl of ribonucleosides in oligonucleotide synthesis.(For example see K. Kikugawa et. al., Chem. Pharm. Bull. 1967, 16, 1110; H. Takaku et. al., J. Org. Chem. 1984, 49, 51; L. W. McLaughlin et. al. , Synthesis, 1985, 322.).

2'-O- Alkyl nucleosides have been prepared by stannous chloride catalyzed reaction of free nucleosides and diazomethane followed by a tedious separation of alkylated isomers as described by M. J. Robins et. al., J. Org. Chem. 1974, 39, 1891. A further alkylation preceedure is described by D. Wagner et. al., J. Org. Chem. 1974, 39, 24. where the free nucleosides uridine ,cytidine and adenosine are alkylated by the reaction of a preformed 2',3'-O-dibutylstannylene nucleoside with alkyl halides to afford a mixture of 2'-O and 3'-O- alkylated products. Alternatively 2'-O-alkylated nucleosides have been

obtained as the result of an exhaustive protection
scheme of both the sugar and /or the heterocycle
followed by selective alkylation of the free hydroxyl
and removal of all the protecting groups. Notable is

5   the use of the 5',3'-O-(tetraisopropyl-disiloxane) as
described by H. Inoue et. al., Nucleic Acids Res.
1987, 15, 6131.,V. A. Gladkaya et. al., Khim. Prir.
Soedin, 1989, 4, 568;   B. S. Sproat et. al., Nucleic
Acids Res. 1989, 18, 41;   T. Akiyama et. al., Bull.

10   Chem. Soc. Jpn. 1990, 63, 3356. as well as tritylation
for this purpose in Y. Furukawa et. al., Chem. Pharm.
Bull. 1965, 13, 1273;   E. Wagner et. al., Nucleic Acids
Res. 1991, 19, 5965; K. Yamana et. al. , Tet. Let.
1991, 32, 6347.   2,2'-Anhydropyrimidines of some

15   common nucleosides are commercially available (Aldrich:
anhydrouridine, anhydrocytidine) or are easily prepared
by those skilled in the art (for example see K. K.
Ogilvie et al., Can. J. Chem., 1969, 47,495; A.
Hampton et. al., Biochemistry, 1966, 5, 2076.).

20   8,2'-Anhydropurines are easily prepared by those
skilled in the art (For a Review of methods see J. G.
Moffatt in " Nucleoside Analogues", R. T. Walker et.
al. ,Eds., Plenum Publishing corp. 1979). K.K. Ogilvie
et al. (1972) Con. J. Chem. 50:2249

25   Metal alkoxides are commercially available and
methods of preparation are known to those skilled in
the art . Meant as an example but not limited to the
following see Gelest Inc., Tullytown, PA, 1994-95
catalogue; Johnson Matthey, ALPHA,Ward Hill, PA,

30   1994-95 catalogue, Aldrich Inc. catalogue. All metal
alkoxid s listed are included herein by reference They

are easily made by reaction of the metal with an excess
of alcohol with optional heating and activation of the
metal (ie. I_2, HgX_2), or reaction of organometalic
compounds with alcohols, or metal hydrides with

5   alcohols , or metal halides with alcohols or alkoxides
(Na,K, other monovalent cation salts) or alcoholysis of
a metal alkoxide with an excess of a second alcohol.

SUMMARY OF THE INVENTION

10   The present invention includes a process for the
production of 2'-substituted nucleosides. The facile
introduction of a large variety of functionalities at
the 2'-position is accomplished via an intramolecular
nucleophilic displacement. 2'-substituted pyrimidines

15   and purines can be made by this method.
The present invention also includes an improved
process for preparing 2'-O-substituted nucleosides. In
this aspect, the invention relates to a process whereby
anhydronucleosides are converted by reaction with a

20   metal (alkoxide)n; preferably where n is at least 2,
to afford the 2'-O-alkyl ribonucleoside. The process
is higher yielding and requires no separation of
isomers, which is an improvement over the prior
processes.

25   Included within the scope of this invention are
2'-modified nucleosides prepared according to the
method of the present invention, phosphoramidites of
the 2'-modified nucleosides, 5'-triphosphates of the
2'-modified nucleosides, and oligonucleotides comprised

30   of at least one of such modified nucleosides.
Nucleosides of the invention can be transformed into

the corresponding 5'-diacylglycero- or
dialkylglycerophosphate derivates for use as prodrugs.
This invention further covers novel nucleosides,
bearing a 2',3' fused heterocyclic substituent,

5   prepared according to the method of the present
invention.

The present invention also includes intramolecular
functionalization of anhydronucleosides at other
positions of the ribose ring.

10   The modified nucleosides of the invention are also
useful as anti-viral and anti-neoplastic agents.

DETAILED DESCRIPTION OF THE INVENTION

A novel and general process is described herein

15   which allows for the facile introduction of a broad
variety of nucleophiles to the 2', 3', 5'-position of
nucleosides. The preferred modification is at the 2' -
position of nucleosides.

A generalized depiction of the reaction step that

20   leads to the 2'-modified nucleosides is show below as
follows:

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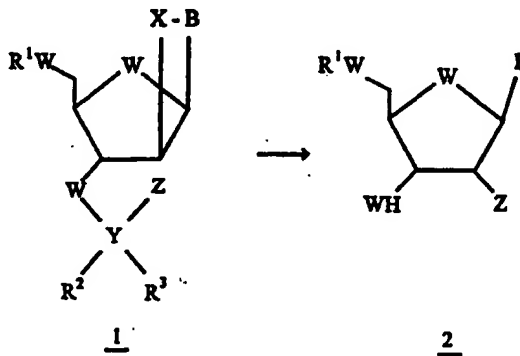
According to this reaction scheme:

B is a nucleobase;

W is independently selected from the group consisting
of O, S, CR^2_2, NR^2, PR^2 and POR^2;

X is selected from the group consisting of O, S, NH,
and NR^4;

Y is selected from the group consisting of a metal,
C, Si, Se, S, B, Al, Sn, and P;

Z is selected from the group consisting of
imidazole, Cl, F, H, ^2H, ^3H, OH, $NHOR^1$, $NHOR^5$,
$NHNHR^5$, NHR^5, =NH, CHCN, $CHCl_2$, SH, SR^5, CFH_2, CF_2H,
$CR^2 2Br$, OR^4 ;

R^1 is selected from the group consisting of H
and an alcohol protecting group;

R^2 is selected from the group consisting of =O,
=S, H, OH, CCl_3, CF_3, halide, optionally
substituted C_1-C_{20} alkyl (including
cyclic, straight chain, and branched), alkenyl,
aryl, C_1-C_{20} acyl, benzoyl, OR^4 and esters;

R^3 is selected from the group consisting of =O,
=S, OH, H, CCl_3, CF_3, halide, C_1-C_{20} alkyl,
alkenyl, aryl, benzoyl, esters, OR^4, omitted, and
cyclopentadiene, cyclooctadiene, CO and
trialkylphosphine if Y is metal;

R^4 is selected from the group consisting of an
optionally substituted hydrocarbon (C_1-C_{20}
alkyl, C_2-C_{20} alkenyl, C_2-C_{20} alkynyl, and
aryl), an optionally substituted
heterocycle, nucleoside, carbohydrate,
fluorescent label, and phosphate;

R^5 is selected from the group consisting of R^2,
R^4, CN, $C(O)NH_2$, $C(S)NH_2$, SO_2R^4, amino acid,

peptide and mixtures thereof.

Other obvious substitutions for components of this reaction scheme are also included within the scope of this invention, which is not limited to the specific, but rather the generalized formula of reaction.

In the preferred embodiments of the invention,

B is selected from the group consisting of a pyrimidine connected to X at the 2-position, a pyrimidine connected to X at the 6-position, and a purine connected to X at the 8-position;

W is O;

X is selected from the group consisting of O, S, and NH;

Y is selected from the group consisting of a metal, C, Si, B, Al, Sn, and P;

Z is selected from the group consisting of imidazole, H, $NHOR^1$, $NHOR^5$, $NHNHR^2$, NHR^2, $=NH$, SH, and OR^4 ;

R^1 is selected from the group consisting of H and an alcohol protecting group;

R^2 is selected from the group consisting of $=O$, $=S$, OH, H, CCl_3, CF_3, halide, C_1-C_{20} alkyl, alkenyl, aryl, C_1-C_{20} acyl, benzoyl, and ester;

R^3 is selected from the group consisting of $=O$, $=S$, H, CCl_3, CF_3, halide, C_1-C_{20} alkyl, alkenyl, aryl, benzoyl, esters and omitted;

R^4 is selected from the group consisting of optionally substituted C_1-C_{20} alkyl, C_2-C_{20} alkenyl, C_2-C_{20} alkynyl, and aryl;   and

R^5 is selected from the group consisting of R^2, R^4 and peptide.

For the purposes of this invention nucleobase will have the following definition. A nucleobase is a purine or pyrimidine base. Nucleobase includes all purines and pyrimidines currently known to those skilled in the art. Nucleobase includes uracil, cytosine, N4-protected cytosine, 4-thiouracil, isocytosine, 5-methyluracil (thymine), 5-substituted uracils, adenine, N6-protected adenine, guanine, N2-protected guanine 2,6-diaminopurine, halogenated purines as well as heterocycles meant to mimic the purine or pyrimidine ring, such as HNCNH. Preferably, the pyrimidine bases are connected to X at the 2 position (2,2'-anhydropyrimidines) or the 6 position (6,2'-anhydropyrimidines); the purine bases are connected to X at the 8 position (8,2'-anhydropurines) or X constitutes the N-3 of the purine (N3,2'-anhydropurines).

As used herein, optionally substituted hydrocarbon refers to groups which consist solely of carbon and hydrogen which may be substituted by groups containing atoms other than hydrogen and carbon. Examples of optionally substituted hydrocarbons are cyanoethyl, allyl, propargyl, methyl, ethyl, propyl, 4-amino butyl, phenyl, napthyl, nitrophenyl, methylphenyl and the like. It is understood that the various substituents must be compatible with standard chemical reactions as would be known by one of ordinary skill in the art.

In certain cases the reaction from 1 to 2 proceeds via the bicyclic intermediate 1a as shown below:

$$\underset{\underline{1a}}{\overset{R^1W \diagdown \overset{W}{\diagup} \diagup B^1}{\underset{R^2 \diagup Y \diagdown R^3}{\underset{W \diagdown \diagup Z}{}}}}$$
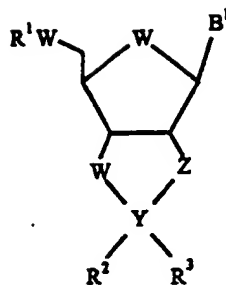
This intermediate may be stable and consists generally of the same chemical functional groups for W, X, Y, Z, R^1, R^2, R^3, R^4, and B as described above, where such compounds are chemically possible.

The cyclization intermediates 1 and 1a as defined above are also included within the scope of this invention, as are all 2'-modified nucleosides 2 that are produced via the intramolecular reaction depicted above. Also included are phosphoramidites and 5'-triphosphates of compound 2, and oligonucleotides comprised of at least one residue consisting of 2. Nucleosides of compound 2 may be transformed by standard methods known to those skilled in the art to the corresponding 5'-diacylglycero- or dialkylglycerophosphate-derivatives for use as prodrugs, among other uses. These modified nucleosides are particularly interesting for antiviral applications. The diacylglycerophosphates of nucleosides and nonnucleosides have been used for modulation of pharmacokinetic behavior, modulation of

bioavailability, and modulation of toxicity as described in United States Patent 5,223,263 which is herein incorporated by reference. Derivatization of the novel nucleosides described in this application is

5 expected to exert similar effects on activity as is true for the diacylglycerophosphates of known nucleoside antivirals such as DDC.

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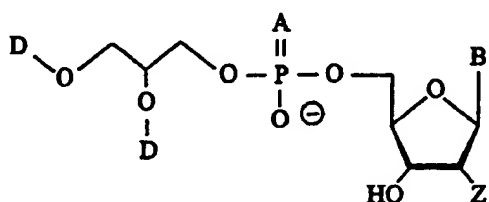
$$D = C(O)(CH_2)nCH_3, (CH_2)nCH_3, \text{where } n = 0 \text{ to } 25$$
$$A = O \text{ or } S$$

15

The cyclization is achieved using bases such as sodium hydroxide, diazabicyclo [5.3.0] undecane (DBU), triethylamine (TEA), diisopropylethylamine (DIPEA),

20 Cs_2CO_3, and the like. Preferably the base is DBU or TEA.

Introduction of a modifying group, which carries an activatable nucleophilic atom in the β-osition to the 3'-oxygen of 5'-protected anhydronucleosides gives

25 intermediates of the general formula 1. These intermediates serve as precursors that can undergo the stereospecific intramolecular introduction of the nucleophile Z to the 2'-position of the nucleoside. The initial cyclization step gives 2',3'-cyclic

30 intermediates 1a. These can be stable compounds, that may have antiviral or anticancer properties. A

preferred intermediate for the production of 2'-NH$_2$
modified nucleosides and triphosphates is the
following:

5
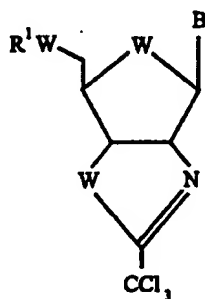
10

This reaction, and subsequent deprotection,
15   proceeds under mild conditions. In many cases, the
resulting 2'-modified nucleosides **2** are novel
compounds. Where Z = NHOH, the heretofore unknown
2'deoxy,2'-N-hydroxylaminonucleosides **3** of the ribo-
configuration are prepared. Where Z = halo methyl, the
20   heretofore unknown 2'-deoxy,2'-halomethylnucleosides **4**
are prepared. Modified nucleosides of the general
formulas **3** and **4** are also included as part of this
invention.

25
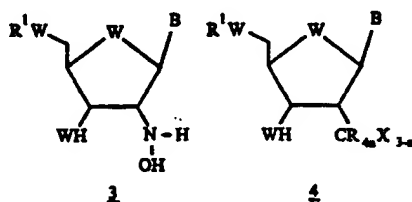
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At the same time, this invention provides a significant

improvement for the preparation of known compounds of the general formula **2**. Such modified nucleosides prepared according to this invention may be transformed by standard methods known to those skilled in the art

5 to the corresponding 5'-triphosphate derivatives. The corresponding mono- and diphosphates are also within the scope of the present invention. The nucleoside triphosphates may also be incorporated into oligonucleotides. In one embodiment, the triphosphates

10 are incorporated by *in vitro* transcription using DNA dependent RNA polymerases. The nucleosides **2** may also be transformed to the suitably protected 3'-phosphoramidite derivatives by standard methods known to those skilled in the art for incorporation into

15 oligonucleotides by automated solid phase synthesis.

In the performance of the process of the present invention, intermediate compound **1** is prepared according to procedures familiar to those skilled in the art. Experimental protocols for the preparation of

20 several examples of intermediate compound **1** are described in the examples below. In the preferred embodiment of the present invention, the intramolecular nucleophilic reaction where compound **1** is converted to compound **2** is accomplished in the presence of Cs_2CO_3

25 and an alcohol. Alcohol protecting group R^1 includes *tert*-butyl diphenylsily (TBDPS), dimethoxytrityl (DMT) any other commonly employed protecting groups, and protecting groups derivatized with Polymeric and solid-phase supports.

30 Intermediate compound **1** may be prepared via a variety of processes. In the preferred embodiment, a

variety of intermediate compound of formula 1 may be prepared from the intermediate compound 3'-O-carbonylimidazole-5'-O-tert-butyldiphenlysily-2-2'-anhydrouridine 5. Such intermediate compound 5 is

5   useful for the introduction of activatable nucleophiles with the correct orientation for the intramolecular reaction of the present invention.

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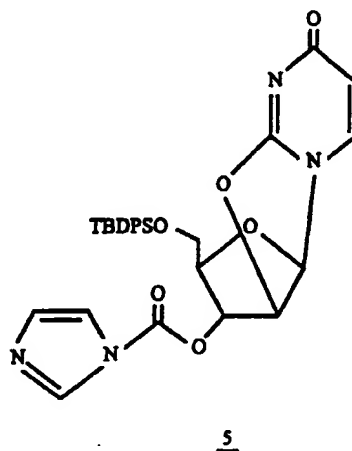
The invention further relates to a process for

20   preparing compounds of the formula 2c which comprises reacting compounds of the formula 1c with a metal alkoxide $M(OR^4)_a$, wherein:

25

1C   2C
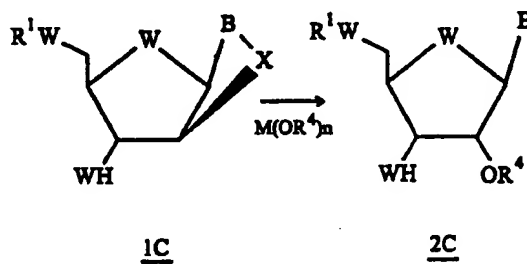
30

B is a nucleobase;

W is independently selected from the group consisting of S, O, CR^2_2, NR^2, pR^2, and POR^2;

X is selected from the group consisting of O, S, NH, and NR^4;

R^1 is selected from the group consisting of H and an alcohol protecting group;

R^4 is selected from the group consisting of optionally substituted hydrocarbon [(C_{1-19}) alkyl, alkenyl, alkynyl, aryl)], optionally substituted heterocycle, nucleoside, fluorescent label, and phosphate.

M is a metal capable of forming a bis or higher alkoxide with OR^4 selected from the group consisting of Mg, Be, Sr, Ba, Th, Zr, Cr, Fe, Ni, Cu, Zn, Mn, Ca, Ce,Ti, Si, Sn, Pd, and the lanthanide series.

n is 2-6.

Alcohol protecting groups are known to those skilled in the art, and include, but are not limited to, trityl groups, substituted silyl groups, etc., H.

More specifically, a preferred embodiment of the invention relates to a process wherein the metal bis alkoxide is formulated with metals exhibiting a +2 oxidation state such as Mg, Ca and the like.

Another preferred embodiment is preparing compounds of formula 2c wherein R' is methyl, propyl, ethyl, butyl, pentyl or allyl.

Another preferred embodiment is the instance where compounds of formula 1c when Y=S are converted to compounds of formula 2c with B now signifying a

2-thiopyrimidine or an 8-thiopurine. Such
compounds(2'-O-substituted 8-thiopurines and the like)
may be desulferized by the use of certain reagents
known to those in the art, for example Raney nickel, to
5   give compounds of formula 2c where B=a purine
unsubtituted at the 8 position.

    Still another preferred embodiment is compounds
of formula 1c wherein X=O,S and B= Uracil, Cytosine,
Guanine, N2-protected Guanine, Adenine, N6-protected
10   adenine.

    The process of the present invention is depicted
in the above scheme. The compounds of formula 1c are
prepared by reaction of a preformed
2,2'-anhydropyrimidine or 8,2'-anhydropurine for
15   example, with a protecting group such as
dimethoxytrityl chloride or t-butyldiphenyl-
chlorosilane and the like in a solvent such as DMF,
pyridine, N-methylpyrrolidinone, dioxane, acetonitrile
Triethyl amine, and the like or mixtures thereof,
20   containing optional additives such as imidazole,
dimethylaminopyridine. The mixture is stirred from
1-24 h between 10-50 °C, preferably at room
temperature. The reaction is evaporated *in vacuo* and
the residue dissolved in an organic solvent such as
25   ethyl acetate or dichloromethane and washed with dilute
aqueous solutions of sodium bicarbonate and/or ammonium
chloride. The organic phase is dried with, for example,
magnesium or sodium sulfate and evaporated. The residue
can be purified by chromatography on Silica gel to give
30   compounds of formula 1c.

    The compounds of formula 2c are prepared by

reaction of compounds of formula 1c with a metal bis alkoxide using from 1-10 equivalents of metal alkoxide in a solvent such as DMF, DMSO, N-methylpyrrolidinone, acetonitrile and the like, preferably DMF. The mixture

5   is heated from 4-24 h between 25-150 °C, preferably at 100 °C. The solvents are removed under vacuum and the residue may be purified by simple extraction procedures or optionally purified by chromatography on silica gel to afford compounds of formula 2c.

10   In the instance where a compound of formula 1c (Y=S) is used to afford a compound of formula 2c (with resultant thiopyrimidine or thiopurine as B) the purine or pyrimidine may be desulferized using reagents known to those skilled in the art, for example refluxing

15   with ethanolic Raney nickel, to afford the compounds of formula 2c where the thio of the purine or pyrimidine has been replaced by a hydrogen.

As shown in the scheme below, various anhydropyrimidines and anhydropurine nucleosides are

20   likely substrates for the method of the invention.

25

30

n,3'-Anhydronucleoside System

5
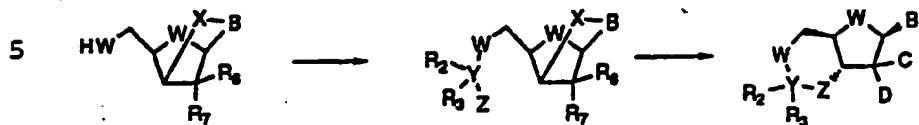
n,5'-Anhydronucleoside System

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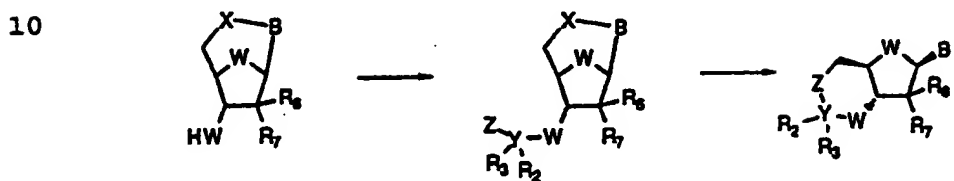
3,3'-anhydropurine system

15
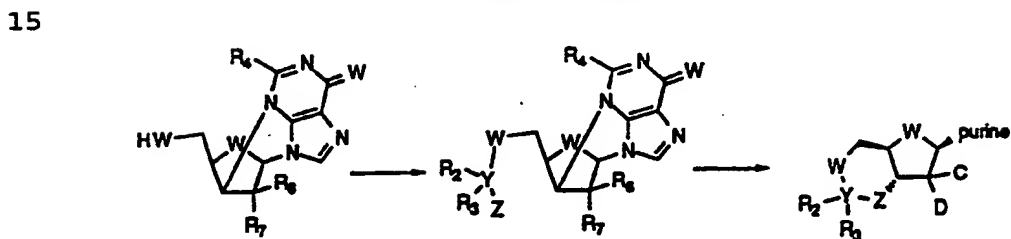
3,5'-anhydropurine system
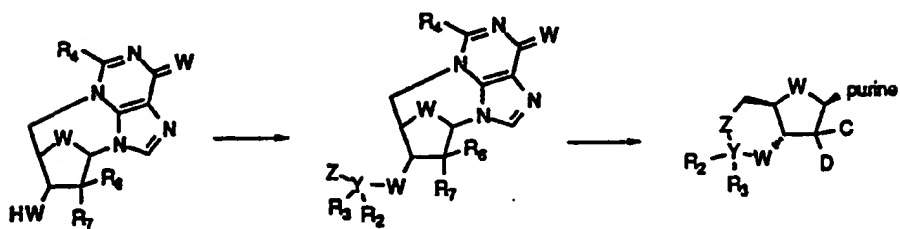
B=nucleobase (purine connected to X
at the 8 position, pyrimidine connected
to X at the 2 or 6 position)
W=O, NH, S, Se, NOR$_1$, NOR$_4$
Y=C, S, Si, P, B, metal, etc...
X=O, NH, S
R$_6$,R$_7$=H, OH, N, OR, O, halogen, etc...

The invention is as broadly described above, and should not be considered to be limited by the breadth of the specific examples below, which serve to illustrate the invention with respect to specific

5   embodiments.

EXAMPLE 1: Preparation of 2'-deoxy-2'-aminouridine.

Uridine is converted to 2,2'-anhydrouridine 6 by standard methodology. (Verheyden et al. (1971) J. Org.

10   Chem. 36:250-254.) Protection of the primary 5'-hydroxyl group gives the 5'-O-(4,4'-dimethoxytrityl) 2,2'-anhydrouridine 7. Intermediate 7 is reacted with trichloroacetonitrile to give the 3'-imidate 8. Typically, the protected anhydrouridine 7 is directly

15   converted to the 2',3'-oxazoline 9. This compound is then hydrolyzed to either the 5'-(4,4'-dimethoxytrityl)-2'-amino-2'-deoxyuridine 10 by treatment with base, or to the fully deprotected 2'-amino-2'-deoxyuridine 11 by treatment with acid. The

20   intermediate oxazole 9 can also be converted to the respective cytidine derivative 12. All NMR where measured at 300 Mhz in DMSO.

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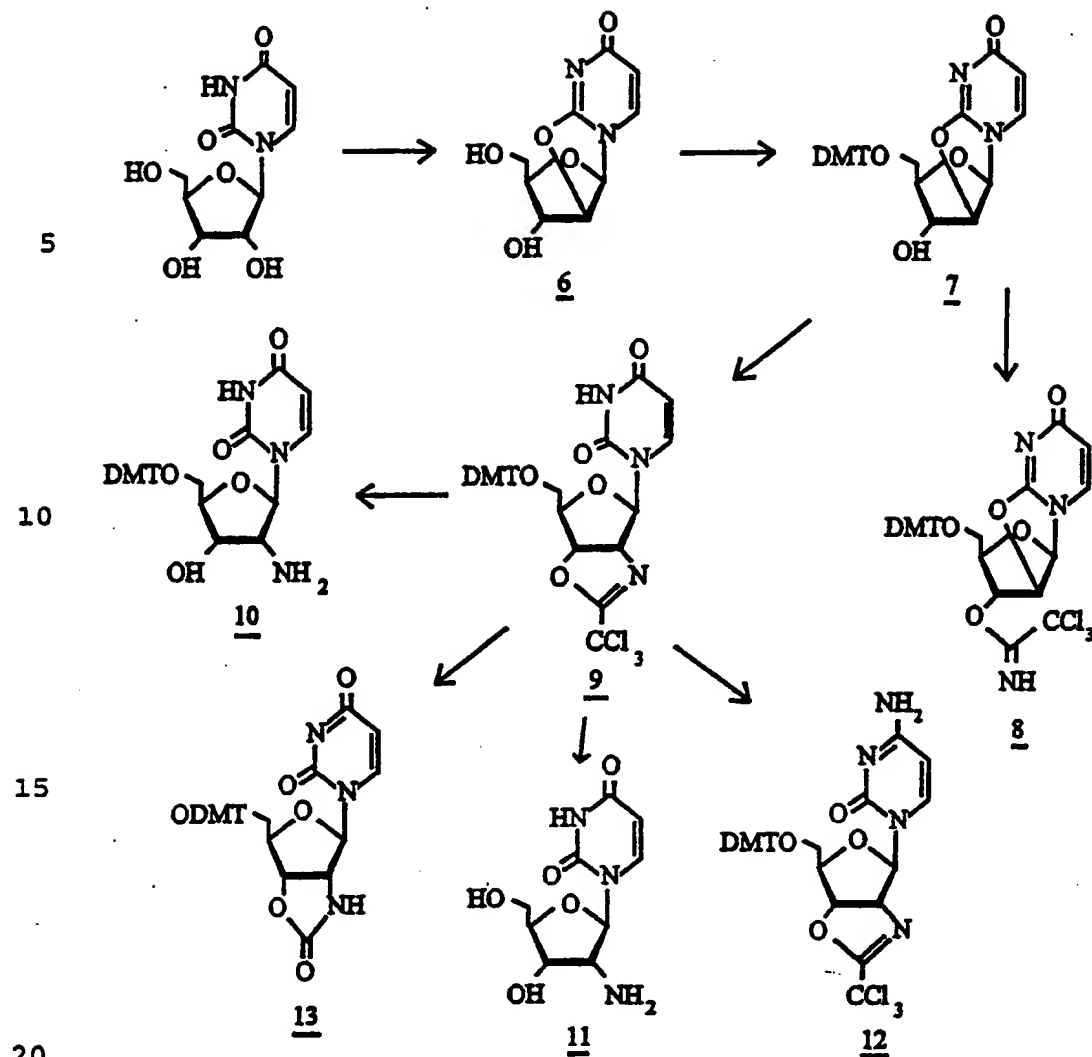
**5'-O-(4,4'-Dimethoxytrityl)-2,2'-anhydro-1-(β-D
arabinofuranosyl)uracil(7).** A suspension of 2,2'-O-
anhydrouridine (10.1g, 0.045 moles) and dimethoxytrityl
chloride (17.5 g, 1.1 eq) in pyridine (100 mL) and
catalytic DMAP (50 mg) was stirred 16hrs at RT prior to
evaporation. The residue was taken up in
dichloromethane, washed with water, followed dil.
sodium bicarbonate. The organic phase was dried with
magnesium sulfate and evaporated. The resulting foam
was purified on silica gel eluting with 0-20%
methanol/ethyl acetate to afford the desired material

as a foam 13.3 g, 56% yield. NMR (DMSO-d_6) Δ 2.81 and
2.85 (ABX, 2 H, H5',5'', J_{ab}= 10.2 Hz, J_{ax}= 4.2 Hz, J_{bx}=
1 Hz), 3.73 (s, 6 H, OCH$_3$), 4.22 (m, 1 H, H3'), 4.31
(m, 1 H, H4'), 5.21 (d, 1 H, H2', J= 5.7 Hz), 5.89 (d,
1 H, H5, J= 7.4 Hz), 5.96 (d, 1 H, 3'-OH , J= 4.4 Hz)
6.33 (d, 1 H, H1', J= 5.6 Hz), 6.84, 7.16, 7.28 (m, 13
H, DMT), 7.96 (d, 1 H, H6, J= 7.4). Anal. calcd for
$C_{30}H_{28}N_2O_7$/0.5H_2O: C,67.03; H,5.43; N,5.21; found: C,
67.02; H, 5.55; N, 4.99.

**5'-O-(4,4'-dimethoxytrityl)-3'-O-(trichloro-
acetimidate)-2,2'-anhydro-1-(β-D-arabino-
furanosyl)uracil (8).** To a solution of 5'-
dimethoxytrityl anhydrouridine 7 (1.0 g, 1.9 mmoles) in
dioxane (5 mL) and trichloroacetonitrile (1 mL) was
added sodium hydride (40 mg, 60% in mineral oil) and
the reaction was stirred 16 h at room temperature prior
to evaporation. The residue was purified on silica gel
eluting with 10% methanol/dichloromethane to afford
imidate 8 as an orange foam (500 mg). NMR (DMSO-d_6) Δ
2.91 and 3.12 (ABX, 2 H, H5',5'', J_{ax}= 4.4 Hz, J_{bx}= 6.3
Hz, J_{ab}= 10.4 Hz), 3.73 (s, 6 H, OCH$_3$), 4.49 (s, 1 H,
H4'), 5.49 (s, 1 H, H3'), 5.55 (d, 1 H, H1', J= 5.7
Hz), 5.95 (d, 1 H, H6, J= 7.5), 6.85 and 7.13-7.29 (m,
13 H, DMT), 7.95 (d, 1 H, H6, J= 7.5 Hz), 10.0 (s, 1 H,
NH).

**5'-O-(4,4'-dimethoxytrityl)-2'-N,3'-O-(2-trichloro-
methyloxazolino)-2'-deoxy-1-(β-D-ribo-
furanosyl)uracil(9).** A mixture of 5'-dimethoxytrityl-
2,2'-anhydrouridine 7 (1.1 g, 2.1 mmoles) in neat

trichloroacetnitrile (5 mL) and sodium hydride (40 mg, 0.5 eq, 60% in mineral oil) was heated at 90°C for 16 h prior to evaporation. The dark residue was purifi d on silica gel eluting with 10% methanol/dichloromethane containing 1% triethyl amine to afford the desired material 9 as a yellow foam (600 mg). NMR (DMSO-d_6) Δ 3.16 and 3.48 (ABX, 2 H, H5' and H5''), 3.72 (s, 6 H, OCH$_3$), 4.14 (m, 1 H, H4'), 5.29 (dd, 1 H, H2', $J_{2',3'}$= 8.3 Hz, $J_{2',1'}$= 1.9 Hz), 5.43 (dd, 1 H, H3', $J_{3',4'}$= 4.5 Hz), 5.65 (d, 1 H, H5, $J_{5,6}$= 8 Hz), 5.92 (d, 1 H, H1', $J_{1',2'}$= 1.8 Hz), 6.86, 7.2, 7.38 (m, 13 H, DMT), 7.84 (d, 1 H, H6, $J_{5,6}$= 8.1 Hz), 11.44 (s, 1 H, NH). C13-NMR (75 MHz) 164.24, 162.44, 159.05, 151.09, 145.67, 144.56, 136.07, 130.47, 130.32, 128.56, 128.33, 127.45, 113.83, 102.37, 93.88, 87.16, 86.79, 86.15, 76.87, 64.31, 55.28, 55.24, 52.05. Anal. calcd for C$_{32}$H$_{28}$N$_3$O$_7$Cl$_3$: C,57.11; H,4.19; N,6.24; Cl,15.80; found: C,57.43; H,4.78; N,6.08; Cl,15.44.

5'-O-Dimethoxytrityl-2'-amino-2'-deoxyuridine (10). Dimethoxytrityl oxazoline 9 (1.5 g, 2.23 mmoles) in dioxane (30 mL) to which is added sodium hydroxide (109 mg in 1 mL water) is refluxed 10 h and then evaporated. The residue was partitioned between water and dichloromethane, dried with magnesium sulfate and evaporated. It was purified on silica gel eluting with 5-10% methanol/dichloromethane to afford first 5'-O-dimethoxytrityl-2'-N,3'-O-(oxazolin-2-one)-2'-deoxyuridine (13) as a yellow foam (900 mg, 58% yield). NMR (DMSO-d_6) 3.15 and 3.36 (ABX, 2 H, H5',5''), 3.7 (s, 6 H, OCH$_3$), 4.20 (m, 1 H, H4'), 4.51 (d, 1 H, H3'),

4.98 (q, 1 H, H2'), 5.59 (d, 1 H, J= 8 Hz, H5), 5.76
(br s, 2 H, H1', OH), 6.67 and 7.23-7.4 (m, 13 H, DMT),
7.68 (d, 1 H, H6, J= 8 Hz), 8.27 (s, 1 H, 2'-NH), 11.47
(s, 1 H, NH). This was followed by the free amino
compound 10 (95 mg) as a foam. NMR (DMSO-d$_6$) Δ 3.18 and
3.22 (ABX, 2 H, H5',5''), 3.38 (m, 1 H, H2'), 3.7 (s, 6
H, OCH$_3$), 3.97 (m, 2 H, H3', H4'), 5.41 (d, 1 H, H5, J=
8 Hz), 5.68 (d, 1 H, H1', J=7.2 Hz), 6.88 and 7.23-7.39
(m, 13 H, DMT), 7.64 (d, 1 H, H6, J= 8.1 Hz). This
product was identical with material prepared via the
traditional 2'-azido route.

2'-Amino-2'-deoxyuridine (11): Dimethoxytrityl
oxazoline 9 (100 mg) was treated with 80% aqueous
acetic acid for 16 h at room temperature and then
evaporated. The residue was co-evaporated with methanol
and then partitioned between dichloromethane/water, the
water evaporated and the residue dried under vacuum to
afford 11 as a glass (50 mg). As sample was
crystallized from MEOH MP 197-199°C (uncorrected). The
product data was identical to published reports.

Example 2: **Preparation of 2'-Hydroxylaminouridine
derivatives:**

14; R=TBDPS

15

16 (33%)   17 (36%)   18 (9%)
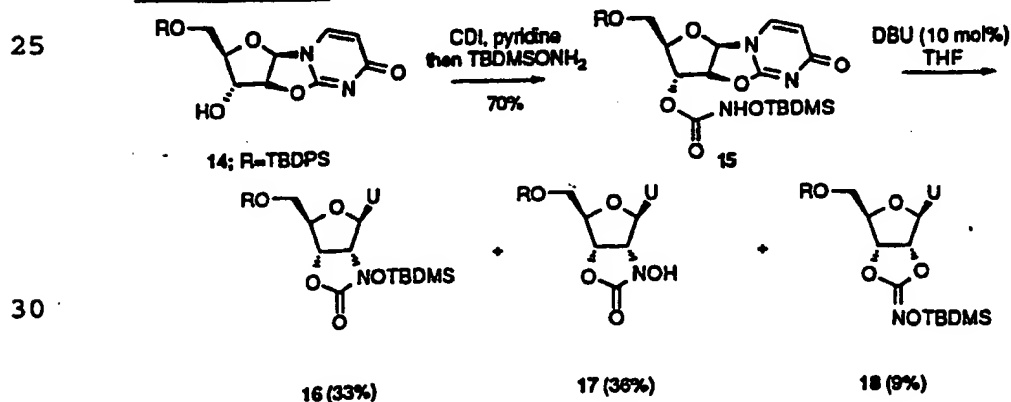
In this example, 5'-O-tert-butyldiphenylsilyl-
2,2'-anhydrouridine (**14**) is converted to the
hydroxylamine derivative **15** by sequential treatment
with carbonyldiimidazole and TBDMSONH₂. This
intermediate containing the latent nucleophile is
exposed to catalytic amounts of base (DBU) and this
results in conversion to three ring-opened
nucleoside products **16**, **17**, and **18**.
2'-Hydroxylamino uridine derivatives **16** and **17**
(which likely results from desilylation of **16**) are a
result of N-selective nucleophilic attack, while
uridine derivative **18** derives from O-selective
nucleophilic attack.

**5'-O-*tert*-Butyldiphenylsilyl-2,2'-anhydrouridine
(14)**: To a stirred slurry of 35 g (0.15 mol) of
2,2'-anhydrouridine in 300 mL of anyhdrous pyridine
and 135 mL anhydrous DMF was added 40.1 mL (0.15
mol) of *tert*-butylchlorodiphenylsilane dropwise via
syringe over 5 min. Upon stirring overnight, all
solids went into solution and the reaction mixture
was concentrated *in vacuo*. The crude residue was
dissolved in 800 mL CH₂Cl₂ and the cloudy solution
washed with 1.2 L NaHCO₃ solution, dried over Na₂SO₄
and concentrated. The residue was recrystallized
from EtOAc to give 45 g (63%) of product as a white
chalk. Concentration of the mother liquor afforded
an additional 4.8 g (6.5%) of crystalline product.
14: ¹H NMR (300 MHz, CDCl₃) d 7.62-7.51 (m, 4H),
7.48-7.30 (m, 7H), 6.17 (d, J=5.8 Hz, H1'), 6.00 (d,
J=7.5 Hz, H5), 5.41 (dd, J=5.9, 1.8 Hz, H2'), 5.26

(d, J=4.8 Hz, OH), 4.61 (m, H3'), 4.26 (dd, J=10.0, 5.2 Hz, H4'), 3.62 (dd, J=11.4, 5.1 Hz, H5'), 3.55 (dd, J=11.4, 6.1 Hz, H5'), 0.98 (s, 9H). ^{13}C NMR (75 MHz, DMSO-d6) d 172.24, 160.68, 137.9, 136.0, 135.9, 133.6, 133.4, 130.9, 128.9, 109.6, 90.2, 89.4, 87.9, 74.7, 63.2, 60.3, 26.8, 19.1.

3'-O-(t-Butyldimethylsilyloxyamino)carbonyl-5'-O-ter t-butyldiphenylsilyl-2,2'-anhydrouridine (15):

5'-O-TBDPS-2,2'-Anhydrouridine (14; 5.0 g, 11 mmol) was co-evaporated with anhydrous pyridine then dissolved in 110 mL of anhydrous pyridine. The flask was flushed with nitrogen, and the 2.3 g (14 mmol) of 1,1'-carbonyldiimidazole was added in one portion as a solid. The solution was stirred at room temperature under nitrogen for 16 hr. A 1 mL aliquot was removed, diluted with 100 mL of ethyl acetate, and washed twice with 100 mL of water. The ethyl acetate layer was dried over sodium sulfate and concentrated *in vacuo* . ^{1}H NMR Analysis indicated complete conversion of starting material. To the reaction mixture was added 2.97 g (19.9 mmol) of O-(t-butyldimethylsilyl)hydroxylamine and the reaction was stirred at room temperature under nitrogen for 5 hr. An additional 0.2 eq (0.32 g) of O-(t-butyl-dimethylsilyl)hydroxylamine was added and the reaction was stirred for 16 hr. The reaction was concentrated *in vacuo* at <30° C and the crude residue dissolved in CH_2Cl_2. The organic phase was washed with $NaHCO_3$ solution, dried over Na_2SO_4, and concentrat d. This material was filtered through

500 mL of silica gel in a sintered glass funnel,
eluting with first 1000 mL hexanes, then with 1000
mL of 50% hexane in EtOAc, then with 500 mL EtOAc,
then 500 mL MeOH/ EtOAc (1:9), and finally with 1000

5   mL 20% MeOH in EtOAc collecting 500 mL fractions.
Concentration of the product containing fractions
afforded 5.12 g (73%) of 15 as a glassy foam. 15: ^{1}H
NMR (300 MHz, d6-DMSO) d 10.46 (br s, NH), 7.93 (d,
J=7.5 Hz, H6), 7.55-7.31 (m, 10H), 6.38 (d, J=5.7

10   Hz, H1'), 5.89 (d, J=7.5 Hz, H5), 5.28 (d, J=5.7 Hz,
H2'), 5.40 (d, J=3.2 Hz, H3'), 4.35 (m, H4'), 3.59
(dd, J=11.3, 5.3 Hz, H5'), 3.50 (dd, J=11.3, 6.4 Hz,
H5'), 0.91 (s, 9H), 0.13 (s, 3H), 0.12 (s, 3H);
Anal. Calc'd for $C_{32}H_{43}N_3O_7Si_2$: C, 60.27; H, 6.79; N,

15   6.59; found: C, 59.16; H, 6.89; N, 6.63.

**Base Catalyzed cyclization of
3'-O-(t-Butyldimethylsilyloxyamino)-carbonyl-5'-O-te
rt-butyldiphenylsilyl-2,2'-anhydrouridine (16, 17,**

20   **and 18):** To a stirred, 23°C solution of 3.0 g (4.7
mmol) of 15 in 45 mL of THF was added 70 mL (0.47
mmol) of DBU. After 16 h, TLC analysis showed
remaining starting material as well as higher Rf
spots. An additional 70 mL of DBU was added and the

25   mixture was stirred 2 h then concentrated *in vacuo* .
Purification of the crude residue by column
chromatography (350 mL of SiO_2 packed in hexanes,
eluting with hexanes, then a gradient of 25-50-75%
EtOAc in hexanes, then EtOAc, and finally 10% MeOH

30   in EtOAc) afforded 16 (27%) as the highest Rf
product as well as regioisomer 18 (9%) as the

intermediate Rf product, and desilylated product **17**
(36%) as the low Rf product. Data for **16**: mp
94-96°C; ¹H NMR (300 MHz, DMSO-d6) d 11.52 (s, 1H),
7.68-7.61 (m, 5H), 7.49-7.41 (m, 6H), 6.11 (d,

5   J=4.56 Hz, 1H), 5.53 (d, J=8.01 Hz, 1H), 5.19 (dd,
J=7.80, 3.69 Hz, 1H), 4.69 (dd, J=7.77, 4.56 Hz,
1H), 4.29 (q, J=4.69 Hz, 1H), 3.91-3.86 (m, 2H),
1.01 (s, 9H), 0.85 (s, 9H), 0.138 (s, 3H), 0.01 (s,
3H); ¹³C NMR (75 MHz, CDCl₃) d 164.3, 158.0, 150.3,

10   141.9, 136.2, 136.0, 133.5, 133.0, 132.7, 130.9,
130.8, 128.7, 128.6, 103.7, 90.6, 86.1, 76.5, 67.6,
63.6, 27.0, 25.7, 19.3, 17.8, 0.5; *Anal.* Calc'd for
$C_{32}H_{43}N_3O_7Si_2$: C, 60.27; H, 6.79; N, 6.59; found: C,
59.11; H, 6.66; N, 6.33.

15

Data for 17: mp 219-220°C; ¹H NMR (300 MHz, DMSO-d6)
d 11.53 (s, 1H), 10.25 (s, 1H), 7.65-7.59 (m, 5H),
7.47-7.36 (m, 6H), 5.90 (br s, 1H), 5.52 (d, 7.95H),
5.17 (dd, J=8.07, 5.55 Hz, 1H), 4.69 (br d, 8.8H),

20   4.19 (q, J=5.3 Hz, 1H), 3.92-3.83 (m, 2H), 0.99 (s,
9H); ¹³C NMR (75 MHz, DMSO-d6) d 164.1, 157.1, 151.0,
143.9, 135.9, 135.8, 133.5, 133.2, 130.7, 128.7,
128.6, 102.7, 91.3, 86.5, 79.6, 67.9, 64.0, 26.7,
18.9; *Anal.* Calc'd for $C_{26}H_{29}N_3O_7Si$: C, 59.64; H,

25   5.58; N, 8.02; found: C, 59.41; H, 5.61; N, 7.85.

Data for 18: ¹H NMR (300 MHz, DMSO-d6) d 11.46 (s,
1H), 7.78 (d, J=8.1 Hz, 1H), 7.63-7.59 (m, 4H),
7.47-7.36 (m, 6H), 5.98 (br s, 1H), 5.74 (br d,

30   J=6.72 Hz, 1H), 5.60 (d, J=7.95 Hz, 1H), 5.39 (dd,
J=6.60, 4.35 Hz, 1H), 4.27 (q, J=5.3 Hz, 1H), 3.92

(dd, J=10.9, 5.1 Hz, 1H), 3.83 (dd, J=10.7, 6.8 Hz, 1H), 0.98 (s, 9H), 0.91 (s, 9H), 0.09 (s, 6H); ^{13}C NMR (75 MHz, CDCl$_3$) d 164.2, 160.4, 150.7, 143.5, 136.3, 136.1, 133.5, 133.2, 130.7, 128.5, 128.4, 103.6, 94.6, 87.1, 86.5, 81.8, 63.8, 26.8, 26.3, 19.2, 18.4, -5.5; *Anal*. Calc'd for C$_{32}$H$_{43}$N$_3$O$_7$Si$_2$: C, 60.27; H, 6.79; N, 6.59; found: C, 59.39; H, 6.93; N, 6.28.

Example 3: <u>Preparation of a 2'-hydroxylaminouridine derivative by catalytic base-promoted cyclofunctionalization, conversion of the nucleoside to the nucleotide triphosphate, and enzymatic modified oligonucleotide synthesis.</u>

14   **19**

20   **21**

T7 RNA polymerase / DNA template → Modified Oligonucleotides
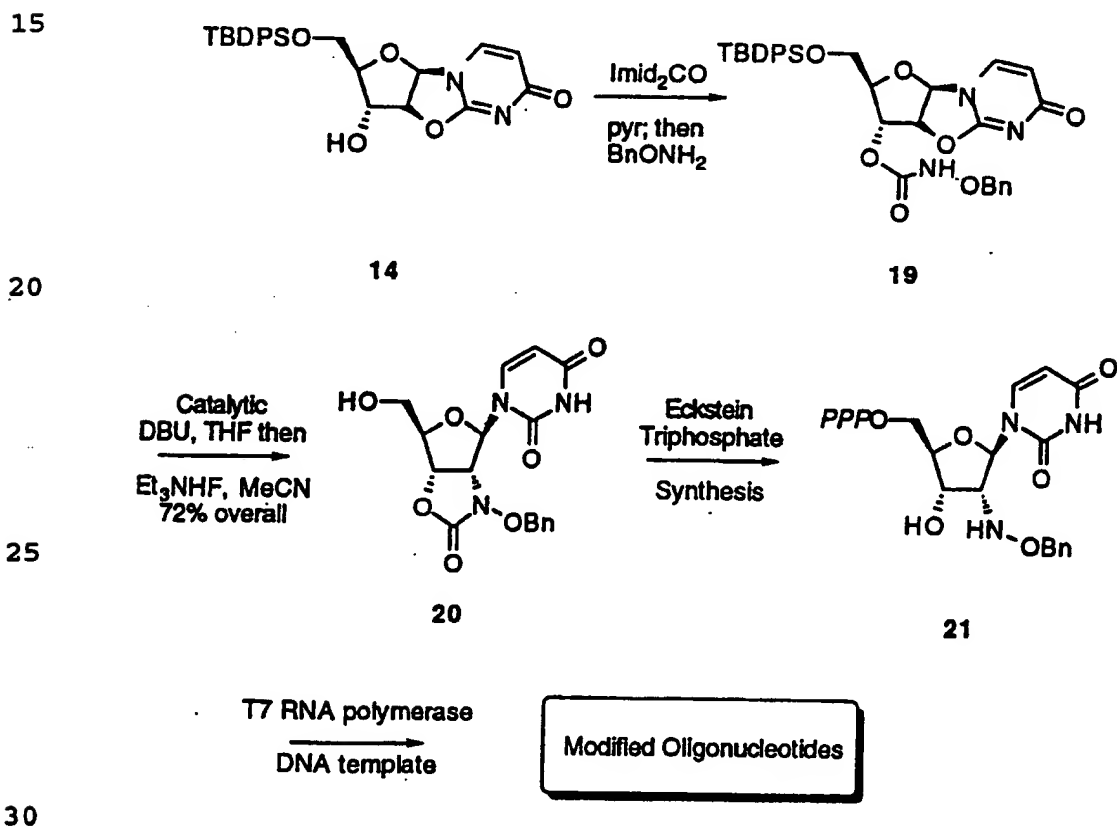
A cyclofunctionalization protocol for the introduction of 2'-NHOR functionality has been developed in which a catalytic amount of base is employed. This scheme has proven suitable for the preparation of the nucleotide triphosphate analog of 2'-deoxy-2'-benzyloxyaminouridine. In the event, 5'-TBDPS anhydrouridine 14 (for preparation, see Example 2) is functionalized by sequential treatment with carbonyldiimidazole and BnONH₂ in pyridine. The cyclization precursor 19 thus prepared is isolated in 93% yield after silica gel filtration. Cyclization is facilitated by treatment of an anhydrous THF solution of the precursor with 10 mole percent of DBU for 24 hours, followed by desilylation of the 5'-OH (TEAHF, MeCN) affords the benzyloxyamine nucleoside analog 21 in 72% overall yield which is suitably derivatized for conversion to the triphosphate. By following a procedure similar to that reported by Ludwig and Eckstein, the corresponding, novel nucleotide triphosphate has been prepared. We found a slight modification of the reported procedure to be preferable in which stoichiometric amounts of the chlorophosphorinone reagent (as reported) and the pyrophosphate solution (vs. 1.5 equivalents of the pyrophosphate utilized by Eckstein) were employed. Furthermore, we found NaIO₄ solution, rather than the reported I₂/ water/ pyridine system, to be a superior oxidant. NH₄OH solution treatment was carried out to cleave the 2'-N,3'-O carbonyl of the substrates. We have found the crude triphosphates prepared in this manner to

be of suitable purity for use in transcription
reactions without further purification by Sephadex.
This 2'-modified triphosphate may have interesting
applications in the SELEX protocol itself, as well

5   as, in principle, serve as a precursor to the known
2'-NH$_2$-2'-deoxy UTP and/ or the novel
2'-NHOH-2'-deoxy UTP.

2'-Amino and 2'-fluoro pyrimidine triphosphates
have been shown to serve as suitable substrates for

10   modified oligo synthesis via DNA template-directed
synthesis with T7 RNA polymerase (Aurup, H.;
Williams, D. M.; Eckstein, F. *Biochemistry*, 1992,
31, 9636. For applications of 2'-NH$_2$ and 2'-F NTPs
in SELEX, see US patent application serial number

15   08/117, 991, filed Sept. 8, 1992 and entitled "High
Affinity Nucleic Acid Ligands containing Modified
Nucleotides"). We have now expanded the scope of
2'-modifications to include the
2'-deoxy-2'-benzyloxyamino UTP derivative.

20   2'-Benzyloxy amino UTP (21) has been incorporated
into modified oligonucleotides via DNA template
directed synthesis with T7 RNA polymerase. While
preliminary transcription studies (employing
standard in house assays for transcriptions of

25   random as well as a fixed sequence DNA templates)
revealed the efficiency of the benzyl derivative to
be 6.8% that of 2'-NH$_2$ UTP at equal concentrations (1
mM), doubling the concentration of benzyloxyamino
UTP to 2 mM improved the relative efficiency to

30   13.3% of that of the amino analog (at 1 mM). The
effect of 2'-NHOBn UTP concentration vs. effeciency

of incorporation was evaluated and a maximum
efficiency of incorporation of approximately 19% of
that of 1 mM 2'-amino uridine triphosphate
incorporation was observed at an analog

5   concentration of 4 to 5 mM. These data are
encouraging for the prospective optimization of the
transcription conditions for this particular
triphosphate, as well as for the potential SELEX
compatibilty of this and other proprietary

10   2'-deoxy-2'-NHOR (including the parent derivative
where R=H) nucleotide triphosphate derivatives.

General Experimental Information: Anhydrous dioxane
and anhydrous pyridine from Aldrich Sure-Seal

15   bottles were used and were sparged with Ar prior to
setting up reaction. 0.5 Molar $(Bu_3NH^+)_2H_2P_2O_7^{2-}$
solution in DMF was prepared according to the
reported procedure (Ludwig, J.; Eckstein, F. *J. Org.
Chem.* 1989, *54*, 631) and was sparged with Ar prior

20   to setting up reaction. ^{31}P NMR Spectra were
measured on samples prepared by dissolving ca 100 mL
aliquots of the reaction mixture in ca 600 mL of
CD_3CN or D_2O. The 5'-OH-nucleoside starting material
was coevaporated with pyridine 2 times in the

25   oven-dried reaction flask directly prior to setting
up the reaction. A new bottle of
2-chloro-4H-1,3,2-benzodioxaphosphorin-4-one was
sublimed at 50° C, transferred to small vials in a
glove bag, and stored in a dessicator at 0° C. In

30   this manner, the reagent may be dissolved in
sparged, anhydrous solvent and transferred via

syringe.   Acceptable purity of the
chlorophosphorinone and pyrophosphate solution was
confirmed by ^{31}P NMR analysis directly prior to
setting up the triphosphate reaction.

5

3'-O-(Benzyloxyamino)carbonyl-5'-O-tert-butyldipheny
lsilyl-2,2'-anhydrouridine (19):   To a stirred, 23°C
solution of 10.0 g (21.5 mmol) of 14 in 40 mL of
pyridine was added 3.67 g (22.6 mmol; 1.05 equiv) of

10   1,1'-carbonyldiimidazole.   The mixture was stirred
until conversion to the corresponding
carbonylimidazole was complete [as determined by [1]H
NMR analysis of concentrated aliquots; ca.12 h:
Signals characteristic of the acyl imidazole

15   intermediate: ^{1}H NMR (300 MHz, CDCl$_3$) d 6.30 (d,
J=5.7 Hz, H1'), 5.98 (d, J=7.5 Hz, H5), 5.73 (br d,
J=2.1 Hz, H3'), 5.55 (br d, J=5.7 Hz, H2'), 4.51 (m,
J=6.5, 2.2 Hz, H4'), 3.68 (dd, J=11.3, 6.0 Hz, H5'),
3.56 (dd, J=11.3, 7.1 Hz, H5'), 1.00 (s, 9H).], at

20   which time 2.9 g (3.72 mmol) of O-benzylhydroxyamine
was added.   The mixture was stirred 3 h,
concentrated *in vacuo*, and purified by filtration
through 1000 mL of silica gel in a sintered glass
funnel (eluting with EtOAc (1 L) then 5% then 7.5%

25   MeOH in EtOAc (1L each), then 10% and 15% MeOH in
EtOAc (2 L each); 800-1000 mL fractions) afforded 12
g (93%) of the product as a white chalk.   Data for
19: mp 107.2-108.8 °C; ^{1}H NMR (300 MHz, CDCl$_3$) d 7.81
(br s, NH), 7.55 (m, 4H), 7.41-7.25 (m, 11H), 7.26

30   (s, 1H), 7.25 (d, J=7.6 Hz, H6), 6.12 (d, J=5.7 Hz,
H1'), 5.93 (d, J=7.5 Hz, H5), 5.43 (br d, J=2.0 Hz,

H3'), 5.29 (br d, J=5.6 Hz, H2'), 4.88 (s, 2H,
OCH₂Ph), 4.32 (dt, J=6.3, 2.1 Hz, H4'), 3.51 (dd,
J=11.3, 6.3 Hz, H5'), 3.50 (dd, J=11.2, 6.4 Hz,
H5'), 1.01 (s, 9H). *Anal.* Calcd for C₃₃H₃₅O₇N₃Si C,
64.58; H, 5.75; N, 6.85. Found C, 63.94; H, 5.81; N,
6.85.

**DBU Catalyzed cyclization of
3'-O-(Benzyloxyamino)carbonyl-5'-O-*tert*-butyldipheny
lsilyl-2,2'-anhydrouridine:**

**2'-Benzyloxyamino-5'-O-*tert*-butyldiphenylsilyl-2'-N,
3'-O-carbonyl-2'-deoxyuridine:** To a stirred, 23°C
solution of 8 g (13.03 mmol) of 19 in 110 mL of THF
(0.12 M) was added 0.2 mL (0.13 mmol) of DBU. After
48 h, the mixture was concentrated *in vacuo* and the
crude residue dissolved in EtOAc. The organic
solution was washed once with saturated NaHCO₃
solution, dried over Na₂SO₄, filtered, and
concentrated. No further purification of the
product was carried out. An analytical sample was
purified by silica gel chromatography (eluting with
hexanes/ EtOAc) ¹H NMR (300 MHz, CDCl₃) d 8.22 (br s,
NH), 7.68-7.56 (m, 4H), 7.49-7.32 (m, 11H), 6.78 (d,
J=8.1 Hz, H6), 5.43 (dd, J=8.1, 1.9 Hz, H5 [w/ D₂O
this signal was observed as a doublet, J=8.1]), 5.16
(d, J=2.1 Hz, H1'), 5.09 (dd, J=8.1, 5.0 Hz, H3'),
5.08 (d, J=11.6 Hz, OCH₂Ph), 5.02 (d, J=11.6 Hz, 1H,
OCH₂Ph), 4.23 (dd, J=8.1, 2.1 Hz, H2'), 4.18 (q,
J=4.7 Hz, H4'), 3.91 (dd, J=11.3, 4.2 Hz, H5'), 3.81
(dd, J=11.4, 4.5 Hz, H5'), 1.02 (s, 9H); ¹³C NMR (75
MHz, CDCl₃) d 164.44, 158.07, 150.40, 142.65, 136.47,

136.28, 136.09, 133.43, 133.04, 130.79, 130.03,
129.68, 128.57, 103.24, 92.09, 86.61, 78.69, 76.43,
67.69, 63.52, 26.89, 19.25. *Anal.* Calcd for $C_{33}H_{35}O_7N_3Si$
C, 64.58; H, 5.75; N, 6.85. Found C, 64.78; H, 5.88;
N, 7.18.

**2'-Benzyloxyamino-2',3'-N,O-carbonyl-2'-deoxyuridine
(20):** The crude 2'-benzyloxyamino-5'-O-*tert*-
butyldiphenylsilyl-2'-N,3'-O-carbonyl-2'-deoxyuridin
e (13.03 mmol) prepared above was dissolved in 50 mL
of MeCN and treated with 1.89 g (15.6 mmol) of solid
Et₃NHF. The mixture was stirred 48 h, at which time
no starting material remained by TLC analysis. The
reaction mixture was concentrated to 1/4 the
original volume and then applied directly to a
column of 400 mL of silica gel packed in EtOAc. The
product was eluted with EtOAc to afford 3.5 g (72%
overall from 19). Data for compound 20: ¹H NMR (300
MHz, CDCl₃) d 11.49 (s, 1H), 7.67 (d, J=8.1 Hz, 1H),
7.42-7.36 (m, 5H), 5.96 (d, J=2.2 Hz, 1H), 5.68 (dd,
J=8.0, 1.5 Hz, 1H), 5.19 (t, J=5.4 Hz, 1H),
5.07-4.99 (m, 3H), 4.68 (dd, J=8.2, 2.2 Hz, 1H),
4.11 (q, J=4.8 Hz, 1H), 3.63 (t, J=5.1 Hz, 2H.

**2'-Benzyloxyamino-2'-deoxyuridine 5'-O-triphosphate
(21):** To a stirred solution of 0.61g (1.64 mmol) of
the nucleoside 20 in 6 mL of pyridine under Argon
was added a solution of 0.35 g (1.72 mmol, 1.05
equiv) of
2-chloro-4H-1,3,2-benzodioxaphosphorin-4-one in 6 mL
of dioxane. After 60 min, ³¹P NMR analysis showed

clean conversion to the 5'-nucleoside phosphorinone (^{31}P NMR, (121.5 MHz, CD_3CN d 131.54, 131.47). To this solution was added 0.39 mL of Bu_3N via syringe followed immediately by addition of 3.25 mL (1.64 mmol, 1 equiv) of the pyrophosphate solution. After 60 min, ^{31}P NMR analysis showed clean conversion to the anticipated cyclic intermediate (^{31}P NMR, CD_3CN d 113.21 (t, J=43 Hz), -13.51 (d, J=43 Hz)). At this stage, several different oxidation conditions were evaluated by treating 2 mL aliquots of the reaction mixture with different oxidation conditions. The cyclic intermediate seems to be relatively stable if stored at 0 °C, although impurity peaks in the $^{31}$P NMR were observed to increase in intensity over 48 h. Oxidation systems studied thus far include the standard I_2/ pyridine/ water system, 70% tBuOOH/ water, solid $NaIO_4$, and 0.1 M $NaIO_4$ solution. The best results (2 separate experiments) to date, as determined by ^{31}P NMR analysis of the crude oxidation reactions, were obtained by employing an aqueous, 0.1 M $NaIO_4$ solution. Treatment of the reaction mixture with 1 equivalent of 0.1 M $NaIO_4$ solution, followed after 30 seconds with excess solid Na_2SO_3 resulted clean transformation to the linear triphosphate intermediate [^{31}P NMR (121.5 MHz, D_2O) d -2.5 (br d), -4.0 (d, J=18 Hz), -14.6 (br t)]. It is noteworthy that chemical shifts and peak resolution observed in ^{31}P NMR spectra of this intermediate, and other triphosphates, varied somewhat from sample to sample, presumably as a result of crude material salt forms or sample

concentration, etc.. . The reaction mixture was concentrated in vacuo at <30 °C, diluted with water, and washed with CH_2Cl_2 until the organic phase was colorless. The water layer was concentrated in

5   vacuo at <30°C to afford a white foam. Examination of the crude material by ^{31}P and ^1H NMR analysis showed remarkably clean 2',3'-protected triphosphate. Treatment of this triphosphate with concentrated NH_4OH for one hour resulted in

10   conversion to the final product **21**. ^1H NMR (300 MHz, D_2O) d 7.81 (d, J=8.1, H6), 7.40-7.29 (m, 5H), 6.15 (d, 7.5 Hz, H1'), 5.89 (d, J=8.1, H5), 4.69 (s, 2H, PhCH_2), 4.61 (m, 1H), 4.27-4.20 (m, 3H), 3.86 (t, J=7.1), 3.20 (q, J=7.3, Et_3NH^+), 1.27 (t, J=7.3,

15   Et_3NH^+): ^{31}P NMR (121.5 MHz, D_2O) d -1.3 (d, J= 19 Hz), -6.5 (d, J= 19 Hz), -17.04 (t, J= 19 Hz). Also observed was a broadsignal at d -1.98.

        EXAMPLE 4: <u>Intramolecular introduction of 2'-alkyl</u>

20   <u>substituents</u>.

The cyclofunctionalization of the 2'-position of 2,2'-anhydrouridine can also be exploited for the introduction of carbon substituents. This is a particular attractive feature of the present

25   invention. No general technology exists in the literature for preparation of 2'-alkyl substituted nucleosides. Cleavage of the initial 2',3'-cyclic intermediates generates functionalized 2'-alkyl substituents which can be derivatized further.
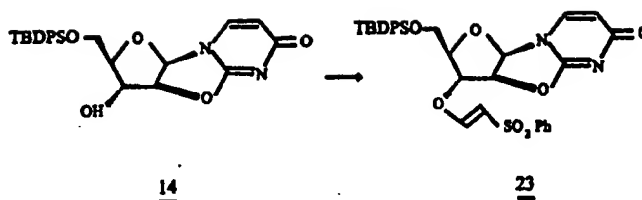
14   22
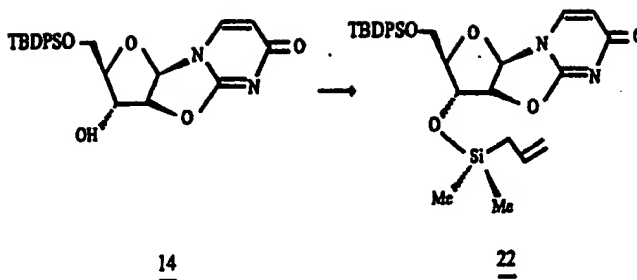
5'-O-*tert*-Butyldiphenylsilyl-3'-O-

(allyldimethyl)silyl-2,2'- anhydrouridine (22): To

a stirred, 0°C solution of 0.5 g (1.08 mmol) of 5'-

O-*tert*-butyldiphenylsilyl-2,2'-anhydrouridine in 4

mL Et_3N/CH_2Cl_2 (1:3) was added 173 mL (1.18 mmol) of

allyldimethylsilyl chloride. The mixture was

allowed to warm to ambient temperature and stirred

16 h. The mixture was concentrated *in vacuo* and

applied directly to a column of 75 mL silca gel,

eluting with EtOAc then MeOH in EtOAc (1:19 then 1:9

then 1.5:8.5) to afford 200 mg (40%) of recovered

starting nucleoside 330 mg (54%) of the higher Rf

product 22. ^{1}H NMR (300 MHz, $CDCl_3$) Δ 7.61-7.57 (m,

4H), 7.46-7.29 (m, 6H), 7.27 (d, H6), 6.14 (d, J=5.8

Hz, H1'), 5.99 (d, J=7.5 Hz, H5), 5.84-5.71 (m,

vinyl H), 5.16 (dd, J=5.7, 1.0 Hz, H2'), 4.96 (br

d,vinyl H), 4.92 (br d, vinyl H), 4.70 (br m, H3'),

4.20-4.13 (m, H4'), 3.51 (dd, J=11.2, 5.3 Hz, H5'),

3.49 (dd, J=11.2, 6.9 Hz, H5'), 0.96 (s, 9H), 0.01

(s, 6H).

5

<u>14</u>   <u>23</u>

10

5'-O-tert-Butyldiphenylsilyl-3'-O-[(E)-2-
(phenylsulfonyl)-ethenyl]-2,2'-anhydrouridine (23):

To a stirred, 0°C slurry of 3.0 g (6.46 mmol) of

15   5'-O-tert-butyldiphenylsilyl-2,2'-anhydrouridine and

2.39 g of trans-1,2-bis(phenylsulfonyl)ethylene

(7.76 mmol) in 30 mL of anhydrous THF was added 196

mg (7.76 mmol) of 95% NaH. The mixture was stirred

under argon and allowed to warm to ambient

20   temperature.

After 24 h, the mixture was diluted with 300 mL

CH_2Cl_2 and the organic solution washed with $NaHCO_3$

solution, dried over Na_2SO_4 and concentrated in

vacuo. Chromatography of the crude residue on 200

25   mL of silica gel eluting with EtOAc then MeOH in

EtOAc (1:19 then 7.5: 92.5 then 1:9) afforded 3.8 g

(93%) of the product as a pale yellow foam. ^{1}HNMR

(300 MHz, $CDCl_3$) Δ 7.80 (d, 2H), 7.78-7.35 (m, H11),

7.25 (d, J=7.5 Hz, H6), 6.29 (d, J=5.8 Hz, H1'),

30   6.05 (d, J=12.4 Hz, vinyl H), 5.91 (d, J=7.5 Hz,

H5), 5.46 (d, J=5.8 Hz, H2'), 4.89 (br s, H3'), 4.42

(m, H4'), 3.58 (dd, J=11.0, 5.5 Hz, H5'), 3.35 (dd,

J=11.0, 8.5 Hz, H5'), 0.97 (s, 9H); ^{13}C NMR (75 MHz,

CDCl$_3$) Δ 172.1, 159.9, 158.3, 142.3, 136.1, 136.0,

135.3, 134.0, 132.9, 132.5, 131.0, 130.1, 130.9,

128.8, 128.7, 127.8, 111.1, 110.9, 90.7, 86.2, 85.2,

83.3, 62.5, 26.8, 19.1.

EXAMPLE 5: Stereospecific reduction of the 2'-position.

The technology for intramolecular introduction of a substituent to the 2'-position of 2,2'-anhydrouridine via an activatable 3'-substituent can also be exploited for stereospecific reduction of the 2'-position. The conversion of the 2,2'-anhydrouridine to 2'-deoxyuridine (reported below) may not be of commercial utility. However, instead of a hydride, a deuterium or tritium label can be introduced to the 2'-position in analogous fashion to give stereospecifically labeled pyrimidine nucleosides.

Red-Al, PhMe
dioxane, 23°C
⟶
24%

7   24
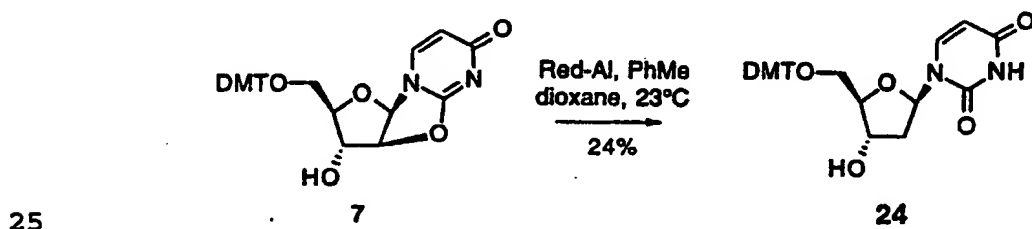
5'-O-Dimethoxytrityl-2'-Deoxyuridine (24). A solution of 5'-dimethoxytrityl anhydrouridine 7 (200 mg, 0.38 mmoles) in dioxane (3 mL) and toluene (9 mL) was added RED.AL (sodium bis(2-methoxyethoxy)aluminum hydride, 136 µl of a 3.4 M solution in toluene) and the reaction was stirred 16

h at room temperature. The reaction was quenched by addition of sodium chloride solution and the phases partitioned, the organic phase washed with sat. ammonium chloride, dried with magnesium sulfate and evaporated. The residue was purified on silica gel eluting with 5-10% methanol/dichloromethane to afford 24 as an oil (50 mg). NMR (DMSO-d_6) Δ 2.19 (t, 2 H, H2', J= 2 Hz), 3.19 and 3.21 (ABX, 2 H, H5',5''), 3.72 (s, 6 H, OCH$_3$), 3.87 (m, 1 H, H4'), 4.29 (m, 1 H, H3'), 5.37 (br d, 2 H, H5, OH), 6.75 and 6.9 and 7.12-7.4 (m, 13 H, DMT), 7.64 (d, 1 H, H6), 11.36 (br s, 1 H, NH).

EXAMPLE 6: <u>5-Bromo-2,2'-anhydrouridine; Preparation and 2'-Derivitization via Intramolecular Nucleophilic Anhydro Ring Openings</u>.

The scope of the general synthetic method for intramolecular nucleophilic opening of anhydrouridine expands to include a 5-substituted anhydrouridine derivative.

5-Bromo-2,2'-anhydrouridine (26) was prepared in 79% yield from 5'-bromouridine 25 upon treatment with diphenylcarbonate and NaHCO$_3$ in DMF at 80°C. 5'-O-TBDPS derivitization was accomplished affording cyclization precursor 27. This substrate was subjected to a modified version of tricholoracetimidate cyclization described in Example 1 (CCl$_3$CN, 1 equiv Cs$_2$CO$_3$; 23°C; 97%) to afford high yields of the desired tricholoromethyl oxazoline 28. Acid promoted hydrolysis of the oxazoline ring provided the 2'-NH$_2$ derivative 29.
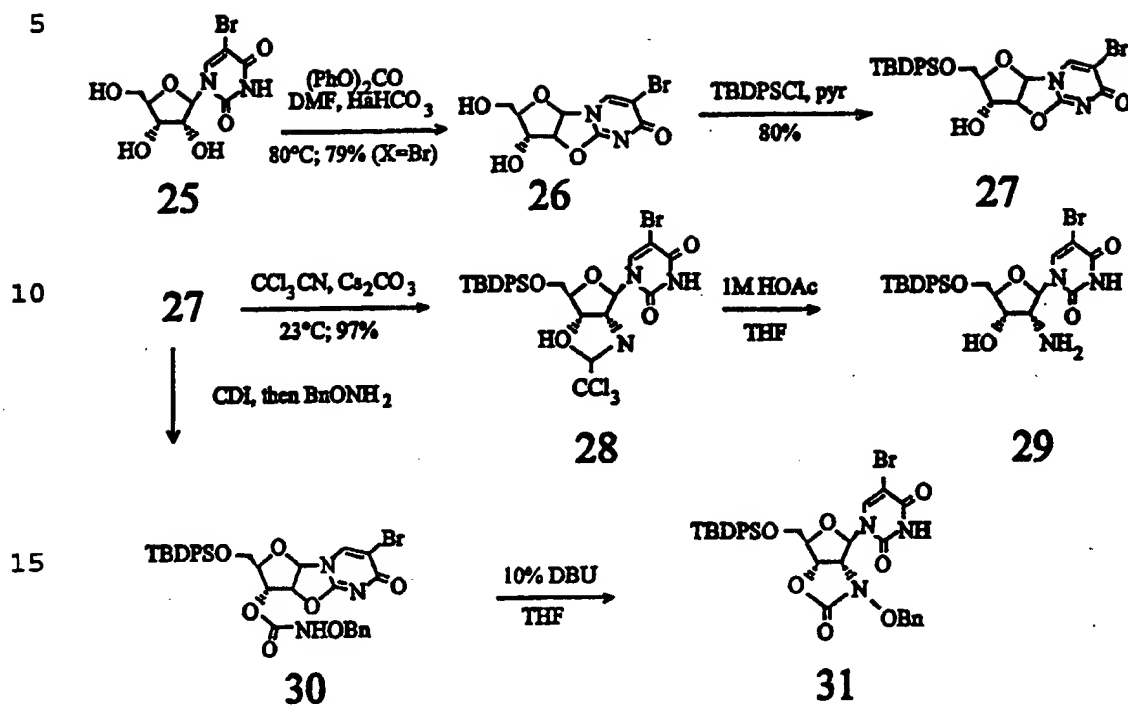
Conversion of **27** to the corresponding
3'-O-carbonylimidazole, followed by benzyloxyamine
conjugation afforded cyclization precursor **30**,
which, upon treatment with 10 mol% DBU in THF
resulted in anhydro ring-opened product **31**.
Products formed by the above processes will be
useful for constructing 5-position modified
nucleosides that are also modified at the
2'-position. Such nucleoside monomers may be useful
precursors to functionally modified oligonucleotides
by either enzymatic synthesis (via conversion to the
nucleoside triphosphate analogues) or automated

synthesis (via conversion to phosphoramidites).
Furthermore, the derived oligonucleotides should be
stabilized toward nucl ase degradation, due to the
replacement of the mechanistically significant

5   2'-hydroxyl by NH_2, NHOMe, or other non-participating
2'-substituents.

5-Bromo-2,2'-anhydrouridine (26): A solution of 1.0
g (3 mmol) of 5-bromouridine (25) in DMF was treated

10   with 0.73 g (3.4 mmol) of diphenylcarbonate and the
mixture was heated to 80°C. After 5 minutes, 25 mg
(0.28 mmol) of $NaHCO_3$ was added. After 2 h, TLC
indicated complete conversion of 25 and the reaction
mixture was cooled to ambient temperature and

15   concentrated *in vacuo* to afford a tan oil. This
residue was dissolved in methanol and the solution
refluxed for 2-3 h. The crude residue was adsorbed
on silica gel and purified by flashing through a
column of silica gel eluting with MeOH/

20   dichloromethane (2:8). Concentration of the product
containing fractions gave 0.65 g (71%) of the
anhydronucleoside as a white foam. ^1H NMR (400 MHz,
DMSO-d6) Δ 8.48 (s, 1H, H6), 6.31 (d, J=5.84 Hz, 1H,
H1'), 5.89 (d, J=4.40 Hz, 1H), 5.23 (d, J=5.84 Hz,

25   1H, H2')., 5.00 (t, J=5.12 Hz, 1H), 4.40 (d, J=4.04
Hz, 1H), 4.13-4.11 (m, 1H), 3.31-3.27 (m, 2H), 3.18
(d, J=5.12 Hz, 1H); ^{13}C NMR (100 MHz, DMSO-d6) Δ
166.42, 160.03, 137.42, 106.34, 91.08, 90.45, 90.25,
75.47, 61.42; Low resolution MS m/e calc'd for

30     $C_9H_9BrN_2O$ (M+): 304.0004, found 304.8.

5-Bromo-5'-O-*tert*-butyldiphenylsilyl-2,2'-anhydro-
uridine (27): To a stirred solution of 7.0 g (23
mmol) of 26 in 20 mL of pyridine was added 6.6 mL
(25.3 mmol) of TBDPSCl. The mixture was stirred at
5   ambient temperature overnight, then concentrated *in
vacuo*. The crude oil residue was dissolved in CH_2Cl_2
and washed with 0.5 N HCl solution (twice), water,
and brine. The crude residue was combined with
another batch prepared in the same manner from 6.8 g
10   (22.3 mmol) of 26 and 6.4 mL (24.5 mmol) of TBDPSCl,
adsorbed on silica gel and purified by flashing
through a column of silica gel eluting with hexanes/
EtOAc (8:2) then EtOAc. Concentration of the
product containing fractions gave 9.93 g (40%) of 27
15   as a white foam. ^1H NMR (400 MHz, DMSO-d6) Δ 8.59 (s,
1H), 7.54-7.40 (m, 10H), 6.33 (d, J=4.38 Hz, 1H),
6.04 (d, J=4.38 Hz, 1H), 5.31 (d, J=4.11 Hz, 1H),
4.44 (br Δ, 1H), 4.21 (m, 1H), 3.63 (dd, J=11.72,
4.4 Hz, 1H), 3.46 (dd, J=11.36, 6.60 Hz, 1H), 0.92
20   (s, 9H); ^{13}C NMR (100 MHz, DMSO-d6) d 166.16, 159.51,
137.41, 135.50, 135.44, 133.05, 132.92, 130.50,
128.50, 106.75, 90.37, 90.08, 88.00, 74.46, 63.23,
26.95, 19.31; Low resolution MS m/e calc'd for
$C_{31}H_{43}BrSiO_5N_3$ $(M+Et_3NH^+)$: 644.255, found 644.1.
25   Analysis calc'd for $C_{25}H_{27}BrN_2O_5Si$: C, 55.25; H, 5.00;
N, 5.16; found: C, 55.02; H, 5.10; N, 5.11.

CCl_3CN Cyclization of 27 to afford 28: A suspension
of 0.27 g (0.5 mmol) of 27 in 2 mL of CCl_3CN was
30   treated with 0.16 g (0.5 mmol) of Cs_2CO_3. The
mixture was stirred at ambient temperature for 4 h

during which time it turned brown, then was concentrated *in vacuo*. The crude residue was filtered through a pad of silica gel to afford 0.33 g (97%) of 28 as a tan solid. ^{1}H NMR (400 MHz, DMSO-d6) Δ 10.05 (s, 1H), 8.62 (s, 1H), 7.53-7.40 (m, 10H), 6.44 (d, J=5.96 Hz, 1H), 5.63 (d, J=2.56 Hz, 1H), 5.59 (d, J=5.96 Hz, 1H), 4.51 (m, 1H), 3.72 (dd, J=11.92, 4.68 Hz, 1H), 3.54 (m, 1H), 0.93 (s, 9H); Low resolution MS m/e calc'd for $C_{33}H_{43}BrCl_3O_5N_4Si$ (M+Et$_3$NH$^+$): 788.1671, found 788.9. Analysis calc'd for C27H27BrCl3N3O5Si: C, 47.14; H, 3.96; N, 6.11; found: C, 46.88; H, 4.02; N, 6.11 .

2'-Amino-5-bromo-5'-O-*tert*-butyldiphenylsilyl-2'-deoxyuridine (29): To a stirred solutiom of 200 mg (0.3 mmol) of 28 in 0.5 mL of THF was added 1 mL of 50% HOAc. After 4 h, the mixture was neutralized by addition of saturated NaHCO$_3$ solution and the aqueous phase extracted with CH$_2$Cl$_2$. The organic phase was dried over Na$_2$SO$_4$ and concentrated. The crude residue was purified by flash silica gel column chromatography (eluting with 6% then 10% MeOH in CH$_2$Cl$_2$) to afford 100 mg (60%) of 29 as a pale yellow solid. ^{1}H NMR (400 MHz, DMSO-d6) Δ 8.59 (s, 1H), 7.56-7.36 (m, 10H), 6.32 (d, J=5.52 Hz, 1H), 6.03 (d, J=4.28, 1H), 5.30 (dd, J=5.08, 1.47 Hz, 1H), 4.45 (br m, 1H), 4.20 (m, 1H), 3.62 (dd, J=11.5, 4.2 Hz, 1H), 3.44 (dd, J=11.08, 3.84 Hz, 1H), 0.91 (s, 9H) .

5-Bromo-3'-O-benzyloxylaminocarbonyl-5'-O-*tert*-butyl

diphenylsilyl-2,2'-anhydrouridine (30): To a
stirred solution of 0.28 g (0.5 mmol) of 27 in 2 mL
of pyridine was added 0.09 g (0.53 mmol) of CDI.
The mixture was stirred overnight, then 0.07 g (0.55

5   mmol) of $BnONH_2$ was added. After 3 days, the mixture
was concentrated *in vacuo* and the residue dissolved
in CH_2Cl_2. The organic phase was washed with
saturated sodium bicarbonate solution, water, and
brine, then concentrated *in vacuo*. The crude

10   residue was adsorbed on silica gel and purified by
flashing through a column of silica gel eluting with
EtOAc. Concentration of the product containing
fractions gave 0.165 g (51%) of 30 as a white foam.
^{1}H NMR (400 MHz, DMSO-d6) Δ 10.89 (br s, 1H), 8.61

15   (s, 1H), 7.55-7.39 (m, 15H), 6.39 (d, J=5.56 Hz,
1H), 5.55 (d, J=5.96 Hz, 1H), 5.39 (d, J=2.96 Hz,
1H), 4.81 (s, 2H), 4.39 (m, 1H), 3.66 (dd, J=11.92,
4.48 Hz, 1H), 3.48 (dd, J=11.48, 6.40 Hz, 1H), 0.92
(s, 9H); ^{13}C NMR (100 MHz, DMSO-d6) Δ 136.2, 135.5,

20   132.9, 130.6, 130.5, 129.5, 128.9, 128.5, 106.5,
90.1, 87.8, 85.7, 76.6, 63.0, 26.9, 19.3.

5-Bromo-2'-benzyloxylamino-2'-N,-3'-O-carbonyl-5'-O-
tert-butyldiphenylsilyl-2'deoxyuridine (31): To a

25   stirred solution of 160 mg (0.229 mmol) of 30 in 3
mL of THF was added 1 drop of DBU (ca 10 mol %).
The mixture was stirred overnight, concentrated in
vacuo and the crude residue purified on flash silica
gel (eluting with 1:1 hexanes-EtOAc) to afford 66 mg

30   (42%) of 31 as a whit foam.

EXAMPLE 7: <u>Intramolecular nucleophilic substitution of the 2'-position of purine nucleosides.</u>

The methodology described for anhydropyrimidine opening is applied to the synthesis of modified purine nucleosides as well. One embodiment would involve nucleophilic opening of a suitably derivatized 8,2'-thioanhydroguanosine such as 34 (a known compound described in Ogilvie et al. (1972) Can. J. Chem. 1100). As shown in the scheme below, any of the examples applied to the anhydropyrimidines above may be employed with such guanosine derivatives.

Example 8: <u>The preparation of 2'-deoxy-2'-methoxylaminouridine via one-step cyclization/deprotection.</u>

In this example, 3'-methoxyaminocarbonyl-2,2'-anhydrouridine (37) is converted directly into the novel uridine nucleoside 2'-deoxy-2'-

methoxyaminouridine (39) via an embodiment of the
method of the invention. Additionally, under the
reaction conditions, further conversion of the
cyclic nucleoside product occurs which results in

5 2'-N, 3'-O-decarbonylation.

In the event, 5'-TBDPS-2,2'-anhydrouridine (14) is
converted to 3'-methoxyaminocarbonyl-2,2'-
anhydrouridine 37 via sequential treatment with

10 carbonyldiimidazole and methoxylamine HCl in
pyridine (93% yield). Upon treatment of 37 with two
equivalents of Cs₂CO₃ in methanol, initial
cyclization to intermediate 38 takes place, followed
by slower conversion to carbonyl deprotected

15 derivative 39 in 68% overall yield from 37.

20
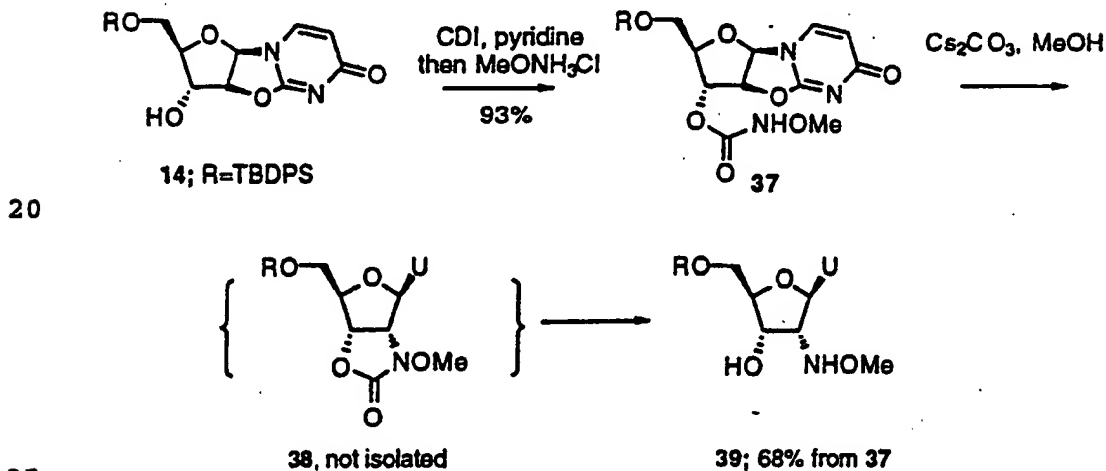
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5'-O-tert-Butyldiphenylsilyl-3'-O-methoxylaminocarbo
nyl-2,2'-anhydrouridine (37): An 0.13 M solution of
5'-O-tert-butyldiphenylsilyl-3'-O-carbonylimidazole-
2,2'-anhydrouridine (14; see Example 2) was prepared

30 from 6 g (12.93 mmol) of 5-O-TBDPS
2,2'-anhydrouridine and 2.5 g (15.5 mmol) of

carbonyldiimidazole in pyridine. To 14 mL (1.83
mmol) of this solution was added 0.17 g (2.04 mmol;
1.1 equiv) of methoxylamine HCl. The mixture was
stirred 3 h, then concentrated *in vacuo*. The

residue was diluted with dichloromethane and washed
with satd NaHCO$_3$ solution, dried over Na$_2$SO$_4$, and
purified by filtration through flash silica gel
chromatography (eluting with EtOAc, then 5% -10%
-15%-20% MeOH in EtOAc) to afford 0.89 g (93%) of

the product as a white solid. 37: ^{1}H NMR (300 MHz,
CDCl$_3$) d 8.88 (br s, 1H), 7.57-7.52 (m, 4H),
7.37-7.30 (m, 7H), 6.28 (d, J=5.6 Hz, 1H), 5.89 (d,
J=7.4 Hz, 1H), 5.45-5.42 (overlapping signals, 2H),
4.36 (dt, J=5.9, 2.7 Hz, 1H), 3.72 (s, 3H), 3.56 (d,

J=5.9 Hz, 2H), 0.98 (s, 9H). ^{13}C NMR (75 MHz, CDCl$_3$) d
171.7, 159.5, 155.6, 135.4, 135.1, 132.78, 132.4,
128.0, 127.9, 127.8, 110.0, 90.1, 87.0, 64.6, 62.8,
26.7, 19.1.

**5'-O-*tert*-Butyldiphenylsilyl-2'-deoxy-2'-methoxylami
nouridine (39):** To a stirred solution of 0.85 g (1.6
mmol) of 2 in 20 mL MeOH was added 1.05 g (2 equiv)
of Cs$_2$CO$_3$. After 20 h, the mixture was concentrated
in vacuo and the residue diluted with EtOAc. The
organic solution was washed with water and dried
over Na$_2$SO$_4$ and concentrated. The residue was
purified by chromatography using100 mL of silica gel
(eluting with a solvent gradient ranging from 10%
EtOAc hexanes to 100% EtOAc) to afford 0.53 g (68%)
of 39 as a white solid. ^{1}H NMR (300 MHz, CDCl$_3$) d
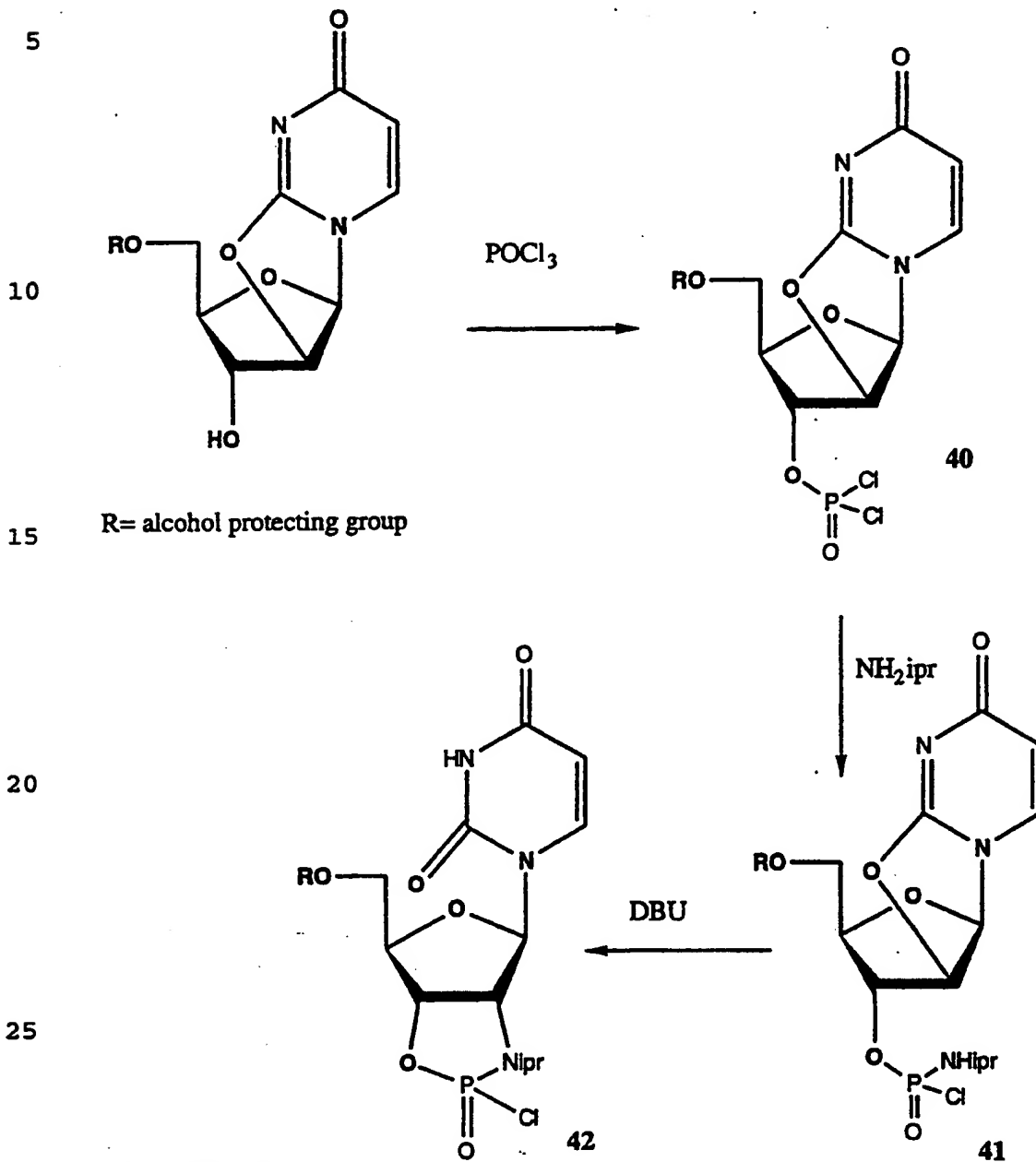7.80 (d, J=8.2 Hz, 1H), 7.65-7.61 (m, 4H), 7.44-7.38

(m, 6H), 6.42 (d, J=5.2 Hz, 1H), 6.01 (d, J=6.7 Hz,
1H), 5.48 (d, J=8.1 Hz, 1H), 4.39 (dd, J=5.5, 2.7
Hz, 1H), 4.15 (br d, J=2.4 Hz, 1H), 3.99 (dd,
J=11.8, 2.2 Hz, 1H), 3.83 (dd, J=11.8, 2.4 Hz, 1H),
3.72 (q, J=5.7 Hz, 1H), 3.60 (s, 3H), 1.07 (s, 9H);
^{13}C NMR (75 MHz, CDCl$_3$) d 163.3, 150.9, 139.8, 135.6,
135.3, 132.6, 132.0, 130.2, 130.1, 128.0, 102.6,
86.8, 85.6, 70.4, 68.1, 64.0, 62.3, 26.9, 19.3.

Example 9.: Preparation of 3'-ox-2'-azaphospholidine derivatives of nucleosides.

The intramolecular introduction of nucleophiles
to the 2'-position of nucleosides also offers an
attractive route for preparation of 2'-modified
3'-phosphate- or 3'-phosphite derivatives. Monomers
of such kind can have application in synthesis of
oligonucleotides with 2'-groups, as well as in
synthesis of backbone modified oligonucleotides.
Cyclic phosphates, in which the phosphorous bridges
the 3'-hydroxyl and the introduced 2'-substituent
could also render a novel class of antiviral
compounds.

The 5'-protected 2,2'-anhydrouridine reacts with
phosphorous oxychloride to give the corresponding
3'-dichlorophosphate derivative. This can be reacted
with an equivalent of a nucleophile futher
activatable for nucleophilic attack, such as
isopropylamine. The latter cyclizes in the presence
of a base such as 10% DBU to yield the nucleoside
3'-ox-2'-azaphospholidine derivative. Chloro
oxazaphospholidine derivatives of ephedrine are well

known stable compounds.[12] Reaction of the latter with
an alcohol such as 2-cyanoethanol gives the
corresponding 2-cyanoethoxyoxazaphospholidine
derivatives.

R= alcohol protecting group

Example 10

A suspension of 2,2'-O-anhydrouridine
(1,10.1g,0.045mol) and dimethoxytrityl chloride
(17.5g,1.1eq) in pyridine (100ml) and catalytic DMAP

(~50mg) was stirred 16 hrs at rt then evaporated .
The residue was washed with dichloromethane/water,
the organic phases washed with dilute sodium
bicarbonate,dried with magnesium sulfate and

5   evaporated. The resulting foam was purified on
silica gel eluting with 0-20% methanol/ethyl acetate
to afford the desired material
5'-O-(4,4'-Dimethoxytrityl)-2, 2'-anhydro-1-(b-D-
arabinofuranosyl)uracil as a foam 13.3g ,56%yield.

10   1H NMR (DMSO) d 2.81 and 2.85 (ABX,2,j_{ab}=10.2 HZ,
j_{ax}=4.2Hz,j_{bx}=~1HZ,H5',5'') ,3.73(s,3,OCH3),
4.22(m,1,H3'), 4.31(m,1,H4'), 5.21(d,1,j=5.7
Hz,H2'),5.89(d,1,j=7.4 Hz,H5), 5.96(d,1,j=4.4
Hz,3'-OH)6.33(d,1,j=5.6 Hz,H1') ,6.84,7.16,

15   7.28(m,13,DMT), 7.96(d,1,j=7.4,H6). Anal. Calcd.
for $C_{30}H_{28}N_2O_7$. $0.5H_2O$: C,67.03; H,5.43; N,5.21.
Found: C,67.02; H,5.55 ; N,4.99.

To a solid residue of Magnesium methoxide
(0.67g, 4equiv., obtained by evaporation of a

20   commercial sample of 10% $Mg(OCH_3)_2$ in methanol to
dryness under reduced pressure on a rotary
evaporator) in a 100 ml round bottom flask was
added 5'-O-(4,4'-Dimethoxytrityl)-2,
2'-anhydro-1-(b-D- arabinofuranosyl)uracil (1.0 g,

25   1.89 mmol) and N,N-dimethylformamide (DMF, 15 ml)
and the mixture was heated 4 hrs at 100 °C. Thin
layer chromatography (TLC) showed the reaction to be
complete. Acetone (ml) was added to the reaction
mixture and filtered. The filtrate was evaporated to

30   dryness , the residue dissolved in ethyl acetate and
washed with water (1X); The solids from the above

filtration were dissolved in water and washed with
ethyl acetate (2X) and the combined organic phases
were dried (MgSO4) and evaporated to afford
5'-O-(4,4'-Dimethoxytrityl)-2'-O-methyluridine
5   (0.94g, 88.7% yield, 95% pure by HPLC). 1-H NMR
identical to a commercial sample.

Example 11
2,2'-O- Anhydrouridine (5.0g, 22.1 mmol),
10   4,4'-Dimethoxytrityl Chloride (8.24g, 24.3 mmol)
and N,N-dimethylaminopyridine (DMAP, ~30 mg) was
dissolved in pyridine (60 ml) and DMF (20 ml) and
the solution stirred 16 hrs at room temperature. The
volume was reduced on a rotary evaporator under
15   reduced pressure (bath temperature< 40 oC). The
residue was diluted with dichloromethane and washed
with dil. sodium bicarbonate (2X), the organic phase
dried (Magnesium sulfate) and evaporated to an oily
residue. The residue so obtained was triturated with
20   ethyl ether (2X). The residue was dissolved in a
small amount of dichloromethane and the product
precipitated with excess ethyl ether (2X) to afford
10.9g of crude 5'-O-dimethoxytrityl-2,2'-
O-anhydrouridine as a yellow powder. This crude
25   product was combined with 4.2 equivalents of
magnesium methoxide (7.56g); (prepared by
evaporation of 90 ml of a 10% Mg(OCH$_3$)$_2$ solution in
methanol to dryness under reduced pressure) in DMF
(400 ml) and the reaction heated 16 hrs at 100 oC
30   and then evaporated. Ethyl acetate was added to the
residue and the solution was washed with dil. sodium

bicarbonate and the aqueous phases back extracted 3X
with ethyl acetate, the combined organic phases
dried (magnesium sulfate), and the solvent
evaporated to afford 5'-O-(4,4'-dimethoxytrityl)-2'

5   -O-methyluridine (12.2g , 98% yield, >90% pure by
1-H NMR).

Example 12
A mixture of 5'-O-dimethoxytrityl-2, 2'-O-

10 anhydrouridine (7) (1.0g, 1.89 mmol), magnesium
n-propoxide (1.62g, 6 equiv.) in DMF (20 ml,
anhydrous) was heated 16 hrs then cooled . Ethyl
acetate (50 ml) was added and the organic phase
washed with dil. sodium bicarbonate, the aqueous

15 phase back extracted twice with ethyl acetate (2X0
the combined organic phases were dried (magnesium
sulfate) and evaporated to dryness. The residue was
purified by chromatography on silica gel eluting
with 20-60% ethyl acetate in hexanes (all containing

20 1% triethyl amine), the appropriate fractions were
pooled and evaporated to afford 5'-O-dimethoxytrityl
-2'-O-propyluridine (0.69g, 62% yield). 1-H NMR d
0.86 (t, 3H, CH3), 1.53 (q, 2H, CH2), 3.25 and 3.3
(ABX, 2H, H-5',5"), 3.53 (dq, 2H, O-CH2), 3.74 (s,

25 6H, DMT), 3.91 (m, 1H, H-2'), 3.98 (m, 1H, H-4'),
4.17 (m, 1H, H-3'), 5.14 (d, J=6.7 Hz, 1H, 3'-OH),
5.29 (d, J=8.1 Hz, 1H, H-6), 5.81 (d, J=3.6 Hz,
1H, H-1'), 6.91 and 7.24-7.40 (m, 13H, DMT), 7.75
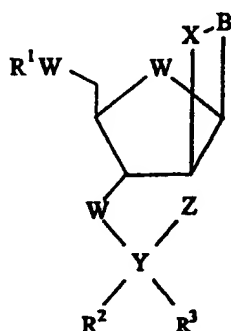(d, J= 8.1 Hz, 1H, H-5), 11.39 (s, 1H, NH).

30

EXAMPLE 13

Calcium allyl alkoxide used was prepared as follows: Calcium hydride was ground to a powder (7g) and then refluxed with 300 ml of allyl alcohol for

5   48 hrs, cooled and filtered and the filtrate evaporated to a sticky solid [Mg(-O-ally)$_2$] which was used as is in the next step. Approx. one half of the above sticky solid was placed in a flask with 5'-O- dimethoxytrityl-2,2'-O-anhydrouridine (1.0g,

10   1.29 mmol) and 30 ml anhydrous DMF and the mixture heated 100 oC 16 hrs colled and evaporated. The residue was dissolved in ethyl acetate and washed with dil. sodium bicarbonate, the organic phase dried (MgSO$_4$) and evaporated. The residue was

15   purified by chromatography on silica gel eluting with 50-80% ethyl acetate in hexanes. The appropriate fractions were pooled and evaporated to afford 5'-O-dimethoxytrityl-2'-O-allyluridine (0.42g, 42% yield). 1-H NMR (300 MHz, DMSO) d 3.24

20   and 3.28 (ABX, 2H, H-5',5"), 3.74 (s, 6H, 2X OCH$_3$), 3.88 (m, 1H, H-2'), 3.97 (m, 2H, allylic CH$_2$), 4.17 (m,1H, H-4'), 4.20 (m, 1H, H-3'), 5.16 and 5.28 (m, 4H, allylic Ha,Hb, H-5, 3'-OH), 5.84 and 5.90 (m, 2H, H-1', allylic Hc), 6.9 and 7.23-7.39 (m, 13H,

25   DMT), 7.73 (d, J=8.1 Hz, 1H, H-6), 11.41 (br s, 1H, NH)

CLAIMS:

1.   A method for the synthesis of 2' modified nucleosides which comprises: a) performing the intramolecular nucleophilic reaction of an

5   intermediate compound having the formula:

10

wherein

15   B is a nucleobase;

W is independently selected from the group consisting of O, S, CR^2_2, NR^2, PR^2, and POR^2;

X is selected from the group consisting of O, S,
        NH, and NR^4;

20   Y is selected from the group consisting of a metal,
        C, Si, Se, S, B, Al, Sn, and P;

Z is selected from the group consisting of
        imidazole, Cl, F, H, ^2H, ^3H, OH, $NHOR^1$, $NHOR^5$,
        $NHNHR^5$, NHR^5, =NH, CHCN, $CHCl_2$, SH, SR^5, CFH_2,

25   CF_2H, CR^22Br, OR^4 ;

R^1 is selected from the group consisting of H and an
        alcohol protecting group;

R^2 is selected from the group consisting of =O, =S,
        H, OH, CCl_3, CF_3, halide, optionally

30   substituted C_1-C_{20} alkyl (including cyclic,
        straight chain, and branched), alkenyl, aryl,

59

C_1-C_{20} acyl, benzoyl, OR^4 and esters;

R^3 is selected from the group consisting of =O, =S,

OH, H, CCl_3, CF_3 halide, C_1-C_{20} alkyl, alkenyl,

aryl, benzoyl, esters, OR^4, omitted, and

5   cyclopentadiene, cyclooctadiene, CO,

trialkylphosphines if Y is metal;

R^4 is selected from the group consisting of an

optionally substituted hydrocarbon(C_1-C_{20} alkyl,

C_2-C_{20} alkenyl, C_2-C_{20} alkynyl, and aryl), an

10   optionally substituted heterocycle, nucleoside,

carbohydrate, fluorescent label, and phosphate;

R^5 is selected from the group consisting of R^2, R^4,

CN, $C(O)NH_2$, $C(S)NH_2$, SO_2R^4, amino acid,

peptide and mixtures thereof; and

15 b)   isolating said 2' modified nucleoside.

2. The method of Claim 1 wherein:

B is selected from the group consisting of a

pyrimidine connected to X at the 2-position, a

20   pyrimidine connected to X at the 6-position,

and a purine connected to X at the 8-position;

W is O

X is selected from the group consisting of O, S,

and NH;

25 Y is selected from the group consisting of a metal,

C, Si, B, Al, Sn, and P;

Z is selected from the group consisting of

imidazole, H, $NHOR^1$, $NHOR^5$, $NHNHR^2$, NHR^2, =NH,

SH, and OR^4 ;

30 R^1 is selected from the group consisting of H and an

alcohol protecting group;

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R² is selected from the group consisting of =O, =S,
OH, H, CCl₃, CF₃, halide, C_1-C_{20} alkyl, alkenyl,
aryl, C_1-C_{20} acyl, benzoyl, and ester;

R³ is selected from the group consisting of =O, =S,

5   H, CCl₃, CF₃, halide, C_1-C_{20} alkyl, alkenyl,
aryl, benzoyl, esters and omitted;

R⁴ is selected from the group consisting of
optionally substituted C_1-C_{20} alkyl, C_2-C_{20}
alkenyl, C_2-C_{20} alkynyl, and aryl; and

10   R⁵ is selected from the group consisting of R², R⁴
and peptide.

3.   The method of claim 1 wherein B is a pyrimidine
base.

15

4.   The method of claim 1 wherein B is a purine
base.

5.   The method of claim 1 further comprising:
20   preparing the phosphoramidite of said 2' modified
nucleoside.

6.   The method of claim 1 further comprising:
preparing the 5'-triphosphate of said 2' modified
25   nucleoside.

7.   The method of claim 1 further comprising:
preparing the 5'-diacylglycerophosphate of said 2'
modified nucleoside.

30

8.   The method of claim 1 further comprising:

preparing an oligonucleotide comprising at least one
of said 2' modifi d nucleosides.

9.   A 2' modified nucleoside prepared according to
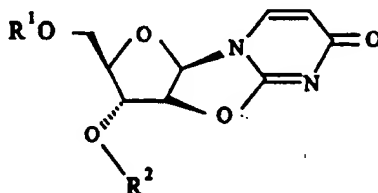the method of claim 1.

10. A phosphoramidite of the 2' modified nucleoside
of claim 9.

11. A 5'-triphosphate of the 2' modified nucleoside
of claim 9.

12. A 5'-diacylglycerophosphate of the 2' modified
nucleoside of claim 9.

13. An oligonucleotide comprising at least one
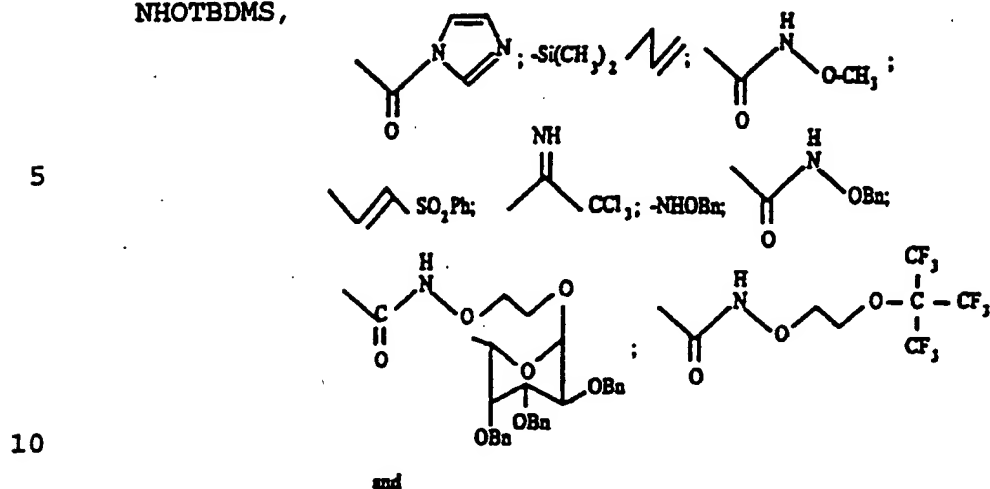nucleotide residue prepared according to the method
of claim 1.

14. A method for the synthesis of 2' modified
pyrimidines which comprises: a) performing the
intramolecular nucleophilic reaction of an
intermediate compound having the formula:

wherein R^1 is H or an alcohol protecting group, and

62

R^2 is selected from the group consisting of - C(O) NHOTBDMS,

b) isolating said 2'-modified pyrimidine.

15. The method of claim 14 further comprising:
preparing the phosphoramidite of said 2' modified
pyrimidine.

16. The method of claim 14 further comprising:
preparing the 5'-triphosphate of said 2' modified
pyrimidine.

17. The method of claim 14 further comprising:
preparing the 5'-diacyl glycerophosphate of said 2'
modified pyrimidine.

18. The method of claim 14 further comprising:
preparing an oligonucleotide comprising at least one
of said 2' modified pyrimidines.

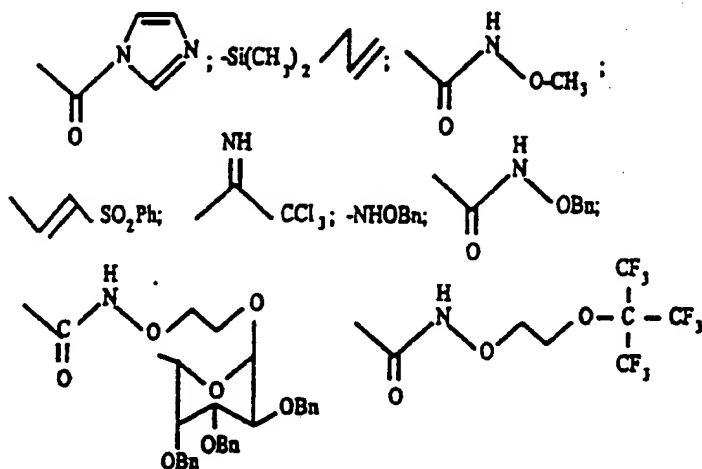19. A modified pyrimidine compound comprised of the
formula:
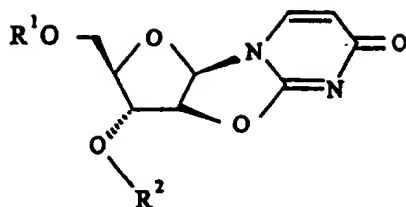
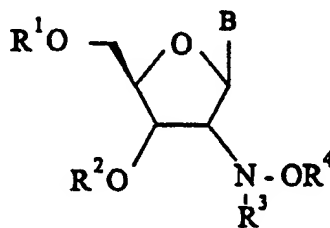wherein R¹ is H or an alcohol protecting group, and R² is selected from the group consisting of -C(O)λ NHOTBDMS,

20. A 2' modified nucleoside comprised of the formula:

wherein B is a pyrimidine or a purine, R¹ and R² are H or an alcohol protecting group, and R³ and R⁴ are H, alkyl, alkenyl, aryl, oligoethylene glycol,

carbohydrate, combination thereof or benzoyl.

21. The 2' modified nucleoside of claim 20 wherein
R^4 is selected from the group consisting of CH$_3$, Bn
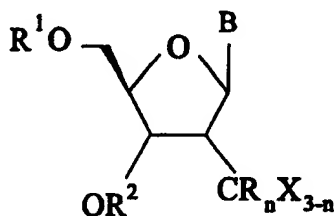5.   and TBDMS and R^3 is CH$_3$.

22. A phosphoramidite of a 2' modified nucleoside
of claim 20.

10   23. A 5'-triphosphate of the 2' modified nucleoside
of claim 20.

24. A 5'-diacyl glycerophosphate of the 2' modified
nucleoside of claim 20.

15

25. An oligonucleotide comprised of at least one
residue of the 2' modified nucleoside of claim 20.

26. A 2' modified nucleoside comprised of the
20   formula:

$$R^1O \underset{OR^2}{\overset{O}{\diagup}} \quad \underset{CR_nX_{3-n}}{\overset{B}{\diagup}}$$

25

wherein B is a pyrimidine or purine, R^1 and R^2 are H
or an alcohol protecting group, X is selected from
the group consisting of F, Cl, Br and I and R is
30   selected from the group consisting of H and
substituted or unsubstituted alkyl.

27.   A phosphoramidite of a 2' modified nucleoside of claim 26.

28.   A 5'-triphosphate of a 2' modified nucleoside of claim 26.

29.   A 5'-diacyl glycerophosphate of a 2' modified nucleoside of claim 26.

30.   An oligonucleotide comprised of at least one residue of the 2' modified nucleoside of claim 26.

31.   A modified nucleoside comprised of the formula:
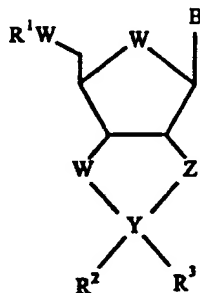
wherein

B is a nucleobase;

W is independently selected from the group consisting of O, S, CR_2^2, NR^2, PR^2, POR^2;

X is selected from the group consisting of O, S, NH, and NR^4;

Y is selected from the group consisting of a metal, C, Si, Se, S, B, Al, Sn, and P;

Z is selected from the group consisting of

66

imidazole, Cl, F, H, ^{2}H, ^{3}H, OH, NHOR^1, NHOR^5,
NHNHR^5, NHR^5, =NH, CHCN, CHCl$_2$, SH, CFH$_2$, CF$_2$H,
CR^{2}2Br, OR^4 ;

R^1 is selected from the group consisting of H and an
5   alcohol protecting group;

R^2 is selected from the group consisting of =O, =S,
H, OH, CCl$_3$, CF$_3$, halide, optionally
substituted C$_1$-C$_{20}$ alkyl (including cyclic,
straight chain, and branched), alkenyl, aryl,
10   C$_1$-C$_{20}$ acyl, benzoyl, OR^4 and esters;

R^3 is selected from the group consisting of =O, =S,
OH, H, CCl$_3$, CF$_3$. halide, C$_1$-C$_{20}$ alkyl, alkenyl,
aryl, benzoyl, esters, OR^4 and omitted;

R^4 is selected from the group consisting of an
15   optionally substituted hydrocarbon(C$_1$-C$_{20}$ alkyl,
C$_2$-C$_{20}$ alkenyl, C$_2$-C$_{20}$ alkynyl, and aryl), an
optionally substituted heterocycle, nucleoside,
carbohydrate, fluorescent label, and phosphate;

R^5 is selected from the group consisting of R^2, R^4,
20   CN, C(O)NH$_2$, C(S)NH$_2$, amino acid and peptide.

32.   The nucleoside of Claim 31 wherein:
B is selected from the group consisting of a
pyrimidine connected to X at the 2-position, a
25   pyrimidine connected to X at the 6-position,
and a purine connected to X at the 8-position;
W is O
X is selected from the group consisting of O, S,
and NH;
30   Y is selected from the group consisting of a metal,
C, Si, B, Al, Sn, and P;

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Z is selected from the group consisting of
imidazole, H, NHOR^1, NHOR^5, NHNHR^2, NHR^2, =NH,
SH, and OR^4 ;

R^1 is selected from the group consisting of H and an

5　　　alcohol protecting group;

R^2 is selected from the group consisting of =O, =S,
OH, H, CCl$_3$, CF$_3$, halide, C$_1$-C$_{20}$ alkyl, alkenyl,
aryl, C$_1$-C$_{20}$ acyl, benzoyl, and ester;

R^3 is selected from the group consisting of =O, =S,

10　　　H, CCl$_3$, CF$_3$, halide, C$_1$-C$_{20}$ alkyl, alkenyl,
aryl, benzoyl, esters and omitted;

R^4 is selected from the group consisting of
optionally substituted C$_1$-C$_{20}$ alkyl, C$_2$-C$_{20}$
alkenyl, C$_2$-C$_{20}$ alkynyl, and aryl; and

15　　R^5 is selected from the group consisting of R^2, R^4
and peptide.

33. A modified nucleoside comprised of the formula:

20

25
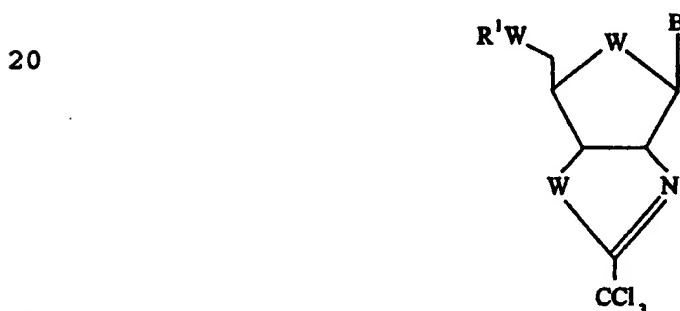
wherein B is a pyrimidine or purine; W is selected
from the group consisting of S, O, CH$_2$, N, and P;and
R is H or an alcohol protecting group.

30　　34. A method for the stereospecific reduction of
the 2, position of 2, 2' anhydro pyrimidine

comprising: performing the intramolecular
introduction of a substituant to the 2' position of
a 2,2' anhydro pyrimidine via an activatable 3'
substituent; and isolating said stereospecifically
5   reduced pyrimidine.

35. A process for preparing a compound having the
formula:

10

$$R^1W \text{---} W \text{---} B$$
$$WH \quad OR^4$$
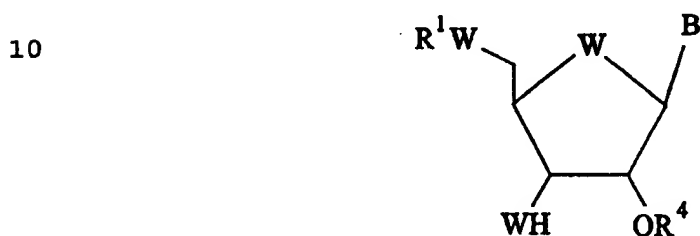
15

comprising reacting a compound having the formula

20

$$R^1W \text{---} W \text{---} B \text{---} X$$
$$WH$$
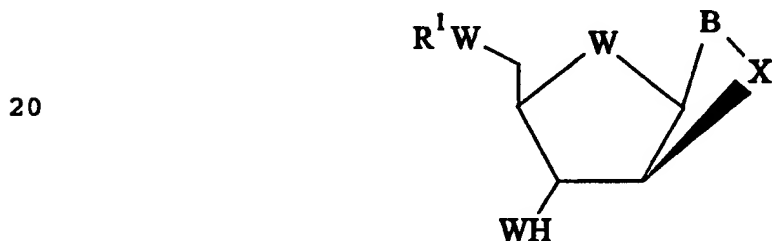
25   with a metal alkoxide , M(OR^4)n$_2$, wherein
        R^1 is selected from the group consisting of H
and an alcohol protecting group;
        W is independently selected from the group
consisting of S, O, CR$^2_2$, NR^2, PR^2, POR^2;
30   X is selected from the group consisting of O,
S, NH, and NR^4;

B is a nucleobase;

R^4 is selected from the group consisting of optionally substituted hydrocarbon [(C_{1-19}) alkyl, alkenyl, alkynyl , aryl)], optionally substituted heterocycle, nucleoside, fluorescent label, and phosphate.

M is a metal capable of forming a bis or higher alkoxide with OR^4 selected from the group consisting of Mg, Be,Sr,Ba,Th,Zr, Cr, Fe, Ni, Cu, Zn, Mn, Ca, Ce,Ti, Si, Sn, Pd, and the lanthanide series.

n is 2-6

36. The process of claim 35 wherein M is selected from the group consisting of calcium, magnesium or cerium.

37. The process of claim 35 wherein B is selected from the group consisting of uracil, cytosine, guanine, and adenine.

38. The process of claim 35 wherein B is a pyrimidine.

39. The process of claim 35 wherein R^1 is selected from the group consisting of dimethoxytrityl or t-butlydipheny silyl.

40. The process of claim 35 wherein R^4 is selected from the group consisting of methyl, ethyl, propyl, allyl, butyl, and pentyl.

41.   The process of claim 35 wherein W and X are
both O.

42.   The process of claim 35 wherein X is S.

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43.   The process of claim 42 which furthur comprises
desulfurizing the compound with a dethiating
reagent.

10   44.   The process of claim 42 wherein B is a purine.

45.   The process of claim 42 wherein B is an
N-protected guanine or adenine.

15   46.   The process of claim 42 wherein B is
2,6-diaminopurine

47.   The process of claim 35 wherein the metal
alkoxide used is magnesium methoxide.

20

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :A61K 31/00 ; C07H 1/00, 19/00, 21/00

US CL :536/28.1, 28.5, 28.53, 28.54

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 536/28.1, 28.5, 28.53, 28.54

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

Please See Extra Sheet.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	Chemical Reviews, Volume 92, Number 8, issued 1992, Donna M. Huryn et al., "AIDS-Driven Nucleoside Chemistry ", pages 1745-1768, see entire document.	1-47
Y	Tetrahedron Letters, Volume 31, Number 4, issued 1990, Jean Tronchet et al. , "Novel Types of Cyclonucleosides", pages 531-534, see entire document.	1-47

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance		
"E"	earlier document published on or after the international filing date	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed	"&"	document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
07 SEPTEMBER 1995	15 SEP 1995

Name and mailing address of the ISA/US	Authorized officer
Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231	JAMES O. WILSON
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B. FIELDS SEARCHED

Electronic data bases consulted (Name of data base and where practicable terms used):

APS on line, search terms : 2(W)modified, 536/clas, oligonucleotide, ph sphoramidite
CAS on line search, structure search in US patent application 08/264,029